EXHIBITS 1 through 10

To the Declaration of Amy Walsh Re:

Mr. Balwani's Motion to Admit Trial Exhibits

EXHIBIT 1

Message

From: Ian Gibbons [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=IGIBBONS]
Sent: 2/18/2010 7:38:39 PM
To: Sunny Balwani [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=Sbalwani]; Elizabeth Holmes [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=Eholmes]
CC: Gary Frenzel [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=Gfrenzel]
Subject: System 4.0 PPT
Attachments: System 4.0.v2.ppt

As requested …

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System 4.0

System component requirements and selection

02/18/2010

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Overview

System 4.0 will be capable of performing any measurement required *in a distributed test setting*

It is envisaged that *several distinct measurement technologies* will be incorporated

The system will be broadly based on the *existing cartridge and reader concepts*

Open architecture for both reader and disposable

The number of total measurements per sample will be increased by 2 to 3-fold (*target: 15 assays?)*

EXHIBIT 2

15004

To: Elizabeth Holmes[/o=theranos organization/ou=first administrative group/cn=recipients/cn=eholmes]; Surekha Gangakhedkar[/o=theranos organization/ou=first administrative group/cn=recipients/cn=surekhag]
Cc: Daniel Young[/o=theranos organization/ou=first administrative group/cn=recipients/cn=dyoung]; Gary Frenzel[/o=theranos organization/ou=first administrative group/cn=recipients/cn=gfrenzel]; Sunny Balwani[/o=theranos organization/ou=first administrative group/cn=recipients/cn=sbalwani]
From: Ian Gibbons[/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=IGIBBONS]
Sent: Tue 10/19/2010 11:23:45 PM (UTC)
Subject: Re: GSK

I think we have demonstrated capabilities fully equivalent to lab. methods in areas where we have done assay development. Our immunoassays match the best that can be done in clinical labs and work with small blood samples. Generally our assays are faster by a factor of three to ten than kits. Our dynamic range capability is more than 10^4 fold, better than any other system. Out sensitivity is state-of-the–art (pM or pg/mL).

We have also shown abilities to work in all assay areas (cytometry, nucleic acids, general chemistry and immunoassay).

In general chemistry, we match reference methods but, importantly, we can use small blood samples.

Cost savings will accrue from small sample size and no blood draw requirement.

Acceleration of studies: Tina just put a Rubella Antibody assay on our system in three days.

We have shown HAI feasibility in less than two months.

On 10/19/10 3:52 PM, "Elizabeth Holmes" <eholmes@theranos.com> wrote:

> Thanks all. I've just landed and will take a look as soon as the materials are circulated. Three points I will want to highlight and quantify as numerically as possible (by how much):
> - why and how our platform will be capable of the full range of assays and be at least as good as the CRO
> - why and how we'll achieve significant cost savings
> - why and how we'll accelerate studies significantly
>
> On Oct 19, 2010, at 2:51 PM, "Surekha Gangakhedkar" <surekhag@theranos.com> wrote:
>
>> Daniel,
>>
>> I will send you the LAMP data in an hour.
>>
>> Thanks,
>> Surekha

From: Daniel Young
Sent: Tuesday, October 19, 2010 2:25 PM
To: Ian Gibbons; Elizabeth Holmes
Cc: Gary Frenzel; Surekha Gangakhedkar; Sunny Balwani
Subject: RE: GSK

We have integrated Ian's IP material into the large slide deck that we have been working on (integrated

from Hopkins and old GSK presentations).

Our efforts to update the 3.X Materials are almost done. We will be finalizing this another 2 hour or so, and then will send out this deck for review to this group.

Next on our plate are putting together the slides updating GSK assay progress:

- I will be working on HAI (I need to do some more analysis to calculate sensitivity/specificity).
- I will integrate in Tina's rubella work
- We are still waiting for LAMP data from Surekha/Gary, and then we will make the slides

We aim to send these additional GSK assay slides by 6pm tonight.

-Daniel

From: Ian Gibbons
Sent: Tuesday, October 19, 2010 2:18 PM
To: Elizabeth Holmes
Cc: Gary Frenzel; Surekha Gangakhedkar; Sunny Balwani; Daniel Young
Subject: GSK

I have put together a PPT for GSK including :

IP
HAI
Rubella Ab assay
LDL separation

The materials are the 3.x folder on the server.
Some HAI materials are hyperlinked into the main PPT because when I tried to paste them into my PPT, the formatting went wrong. Gary and Surekha have been able to access the materials and to fix some problems.

Daniel has the IP files describing Image analysis and centrifugation which I think are in presentable form. The general chemistry data and presentation materials are also in the 3.x folder under Presentations (please use the most recent). Note that we used the term LAMP in that PPT and this reference should be removed.

Daniel also has images and some analysis of Kwesi's urinalysis work (it's not yet clear that we can get a presentable calibration, but there is a dose-response for some 10 analytes: very good news).

I am having some difficulty saving files on the server because:
The files are very large and my internet connection through Comcast may be unreliable. Ian and Nathan have been very helpful.

Please recognize the remarkable work Tina did in cranking out a Rubella antibody assay in a very short time. She went the extra mile for the company and then some.

I will be available by 'phone as needed tomorrow and Thursday. Paul has a heads-up that you may want him to participate if technical matters on the HAI assay need to be addressed.

EXHIBIT 3

Message

From: Daniel Young [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=DYOUNG]
Sent: 4/21/2013 12:18:05 AM
To: Sunny Balwani [/O=THERANOS ORGANIZATION/OU=First administrative group/cn=recipients/cn=sbalwani]
CC: Elizabeth Holmes [/O=THERANOS ORGANIZATION/OU=First Administrative Group/cn=Recipients/cn=eholmes]
Subject: RE: assays for fda filing

For the top 75 whole blood tests, only the following are still under development.

- Troponin I
- Malaria

The following still need final integration on the device:

- CBC/WBC
- PT / aPTT
- Blood typing (rh/abo)
- Blood gases

The flu tests (A, B, H1N1 2009, H1N1 seasonal, H3N2) and step A are 95% completed. They are still completing the pre-validation before moving the tests to CLIA for validation. Progress on the next set of assays is moving well – the primer design algorithm has been enhanced to yield a much higher success rate which is making screening more efficient.

We plan to start running TNAA tests on the 4s device next week. And also we will be testing the new sample extraction process on the device.

-Daniel

From: Sunny Balwani
Sent: Friday, April 19, 2013 10:04 PM
To: Daniel Young
Cc: Elizabeth Holmes
Subject: RE: assays for fda filing

Just the gc, elisa and cyto in while blood.

Did Pranav finish all 6 of his flu assays from march? I know he said he finished 4 by 3/31 and was pushing th get the other 2 in first week of april.

From: Daniel Young
Sent: Friday, April 19, 2013 10:01 PM
To: Sunny Balwani
Cc: Elizabeth Holmes
Subject: RE: assays for fda filing

Hi Sunny, just to make sure, do you mean the first 75 assays which span general chemistry, ELISA, cytometry in whole blood? Or the TNAA blood tests?

Thanks,
Daniel

From: Sunny Balwani
Sent: Friday, April 19, 2013 7:17 PM

To: Daniel Young
Cc: Elizabeth Holmes
Subject: assays for fda filing

Are all the assays for our first blood cartridge complete?

EXHIBIT 4

To: Sunny Balwani[/O=THERANOS ORGANIZATION/OU=First administrative group/cn=recipients/cn=sbalwani]
From: Suraj Saksena
Sent: Mon 7/27/2015 10:17:30 PM
Importance: Normal
Subject: FW: Suraj Saksena: Clinical Lab Director application
Received: Mon 7/27/2015 10:17:32 PM
SS.Notification.ltr.27July2015.doc

Hi Sunny,

Please see below.

I am VERY pleased to see that NRCC is now on their list of approved boards for Clinical Chemist. Apparently, this change was made in 07/2015.

They are asking me to submit a hard copy of my PhD dissertation and also my transcripts. My transcripts have already been sent to LFS, so I don't understand what the issue is there. I will fix a time with Ruby today to talk over the phone and sort this out.

Thanks,
Suraj

From: Garcia, Ruby (CDPH-OSPHLD-LFS) [mailto[redacted]]
Sent: Monday, July 27, 2015 12:06 PM
To: Suraj Saksena
Cc: Afolayan, Dolapo (CDPH-OSPHLD-LFS); Thomas, Robert (CDPH-OSPHLD-LFS); Amad, Zahwa (CDPH-OSPHLD-LFS)
Subject: FW: Suraj Saksena: Clinical Lab Director application

Hi Dr. Saksena,
LFS only reviews complete application with complete requirements. See attached information.

See below the updated ABCC certification approved for the Clinical Chemist Director application.

List of Approved Certifying Organization Examinations

Master's and Doctoral Degree and Director-Level Scientist Licensure **Name of Organization**	**Category**	**Initial Approval Date***	**Contact Information**	**Website**
American Board of Bioanalysis (ABB)	Clinical laboratory bioanalyst	10/12005	906 Olive St, #1200 St. Louis, MO 63101 (314) 241-1445	www.aab/American_Board_of_Bioanalysis.asp
American Board of Clinical Chemistry (ABCC)	Clinical chemist	1/1/2005	1850 K St NW, #625 Washington, DC 20006 (202) 857-0717	www.abclinchem.org
National Certified Registry of Chemists (NRCC)	Clinical chemist	12/10/2008	125 Rose Ann Lane West Grove, PA 19390 (610) 322-0657	www.nrcc6.org
National Certified Registry of Chemists (NRCC)	Clinical toxicologist	3/1/2004	125 Rose Ann Lane West Grove, PA 19390 (610) 322-0657	www.nrcc6.org

American Board of Medical Microbiology (ABMM)	Clinical microbiologist	6/1/2005	1752 N St NW Washington, DC 20036 (202) 942-9257	www.asm.org/index.php/certification/abmm
American Board of Histocompatibility and Immunogenetics (ABHI)	Clinical histocompatibility director	None	P.O. Box 19173 Lenexa, Kansas 66285 (913) 895-4602	www.ashi-hla.org
American Board of Medical Genetics and Genomics (ABMGG)	Clinical cytogeneticist	None	9650 Rockville Pike Bethesda, MD 20814 (301) 634-7315	www.abmgg.org/
American Board of Medical Genetics and Genomics (ABMGG)	Clinical genetic molecular biologist	None	9650 Rockville Pike Bethesda, MD 20814 (301) 634-7315	www.abmgg.org/

Sincerely,
Ruby

Ruby V. Garcia
Office Technician
Personnel Licensing Department
Laboratory Field Services
California Department of Public Health
[redacted]
Email Ad: [redacted]

From: Suraj Saksena
Sent: Saturday, July 25, 2015 6:12 PM
To: Garcia, Ruby (CDPH-OSPHLD-LFS)
Cc: Thomas, Robert (CDPH-OSPHLD-LFS); Obeso, Martha (CDPH-OSPHLD-LFS)
Subject: RE: Question about application

Hi Ruby,

I applied with CDPH- Laboratory Field Services for a Clinical Laboratory Director licensure (Unique Identification number: 1009092; Applicant name: Suraj Saksena). I applied on 05/20/2015 and submitted a signed copy of the attestation form on 05/26/2015 (Please see below my correspondence with you regarding the application).

It has been close to 9 weeks since my application was submitted. Can you please let me know by when can I expect to hear from you regarding the approval of my application. Please let me know if there is anything I can do to help expedite the process or if there is any information required from my end.

Thanks a lot Ruby!

Best
Suraj

From: Garcia, Ruby (CDPH-OSPHLD-LFS) [[redacted]]
Sent: Friday, May 22, 2015 2:43 PM
To: Suraj Saksena
Cc: Thomas, Robert (CDPH-OSPHLD-LFS); Obeso, Martha (CDPH-OSPHLD-LFS)
Subject: RE: Question about application

Hi, see below the full address of LFS/Personnel Licensing. Thank you

Attn: Personnel Licensing
CDPH- Laboratory Field Services/Personnel Licensing
850 Marina Bay Parkway, Bldg. P., 1st Floor
Richmond, CA 94804-6403

Ruby V. Garcia
Office Technician
Personnel Licensing Department
Laboratory Field Services
California Department of Public Health
[redacted]
Email Ad: [redacted]

From: Thomas, Robert (CDPH-OSPHLD-LFS)
Sent: Wednesday, May 13, 2015 2:36 PM
To: Garcia, Ruby (CDPH-OSPHLD-LFS); Obeso, Martha (CDPH-OSPHLD-LFS)
Subject: FW: Question about application

From: LFSpersonnel (CDPH-LFS)
Sent: Wednesday, May 13, 2015 8:23 AM
To: Thomas, Robert (CDPH-OSPHLD-LFS)
Cc: Garcia, Ruby (CDPH-OSPHLD-LFS); Obeso, Martha (CDPH-OSPHLD-LFS)
Subject: FW: Question about application

From: Suraj Saksena [mailto:ssaksena@theranos.com]
Sent: Tuesday, May 12, 2015 8:56 PM
To: LFSpersonnel (CDPH-LFS)
Subject: Question about application

Hi,

I am filling an online application for Clinical Laboratory Director Licensure. The website says that I need to have my University registrar send my official transcript directly to LFS at the address:

California Department of Public Health
Laboratory Field Services (LFS)
Attn: Personnel Licensing Section
850 Marina Bay Parkway, Bldg. P.
Richmond, CA 94804

Separately, under the FAQ (Director) tab on the website, I found information stating that all required documents such as official transcripts must be sent directly to LFS at the following address:

Attn: Director Program
CDPH- Laboratory Field Services
850 Marina Bay Parkway, Bldg. P., 1st Floor
Richmond, CA 94804-6403

Can you please confirm which address I need to have my transcripts and verification of training and experience sent to?

Looking forward to a prompt response.

Thanks,
Suraj

Suraj Saksena, Ph.D.
Director, Assay Systems
Theranos
1701 Page Mill Rd, Palo Alto, CA 94304
P: (650) 823-2848

EXHIBIT 5

Message

From: Elizabeth Holmes [/O=THERANOS ORGANIZATION/OU=FIRST ADMINISTRATIVE GROUP/CN=RECIPIENTS/CN=EHOLMES]
Sent: 12/15/2009 7:32:42 PM
To: thomas.breuer@gskbio.com
CC: Sunny Balwani [sbalwani@theranos.com]
Subject: Follow up to our meeting

Dear Thomas,

It was great to meet you.

In follow up to our conversations, I have attached three documents to this email.

The first is a consolidated summary of the GSK infrastructure we've designed in follow up to our interactions with people on the corporate side in information systems and strategy. We took ten slides on the applications in Biologicals and added them to the end of that summary – slides 28-38. The first slide highlights the ability to use the existing surveillance infrastructure to rapidly test the efficacy of existing vaccines against drifted strains of influenza virus using Theranos' strain-specific real-time antibody tests and the formulas we've established for the relationship between dose, antibody levels, and clinical outcomes.

The second is a copy of the validation report from the GSK staff who tested Theranos technologies in RTP. As you can see in that attachment, GSK's lab Director concluded that "Theranos Systems eliminate the need for a lab." The report shows the ability to get better sensitivity and real-time data using Theranos.

The third is a copy of a case study on Theranos' analytics also reviewed by GSK staff in detail during their due diligence process. This review focused on the ability to improve probability of success of realizing a target product profile with Theranos analytics. The case study details another company's use of Theranos analytics in registrational studies where the system increased POS from 15-80% and saved 18-24 months in clinical development timelines.

The Theranos Solution is a fully integrated and automated system for data capture, analysis, and care delivery. The data capture capability in combination with the predictive analytics capability has been the key to our success in accelerating development timelines.

We are very much looking forward to following up with your clinicians in Philadelphia. Is there a convenient time this week we could meet or arrange a video-conference? Please let us know how best to follow up.

Kind regards,
Elizabeth.

Elizabeth Holmes
President and CEO
Theranos, Inc.

Tel: 650.470.6111
Fax: 650.838.9804

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GTS

GSK's Strategic Enterprise Infrastructure

This presentation and its contents are Theranos proprietary and confidential.

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Contents

- Background
- GTS ROI
- GTS Deliverables
- GTS in Biologicals

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Introduction to Theranos, Inc.

Theranos is a Silicon Valley-based healthcare company founded in 2003.

- Theranos provides fully customized solutions that impact a diverse range of stakeholders in health care by providing actionable information far earlier than historically possible

- Our current and past clients include 9 of the top 15 major pharmaceutical companies, midsized bio-pharmas, prominent research institutions and U.S. and foreign government health organizations

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About Theranos

Founder and CEO Elizabeth Holmes left Stanford University to start Theranos around her patents for next-generation healthcare systems. She has built the company from inception to rapid commercial growth today.

Vice Chairman Sunny Balwani joined Theranos after leaving Microsoft to successfully build and sell his own company for over $400M

Other Management Team Members:

- Dr. Channing Robertson, Dr. Seth Michelson, Jodi Sutton, Dr. David Lester, Dr. Marc Thibonnier

Theranos' investors and board members include, amongst others:

- Donald L. Lucas, the first venture capitalist in Silicon Valley, and a legend behind many of today's Fortune 500 companies
- Larry Ellison, Founder and CEO of Oracle Corporation
- Bob Shapiro, former CEO and Chairman of Monsanto and Pharmacia Corporations (now Pfizer); former director of NYSE, Citibank, and other major corporations
- Draper Fisher Jurvetson; ATA Ventures (spin-out of Institutional Venture Partners)

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Theranos & GSK

- GSK completed a comprehensive validation of Theranos Systems in 2008
  - Validation was independently conducted run by GSK staff at RTP
  - Validation concluded "Theranos Systems eliminate the need for a lab"

- Over the past four years, leads from all three business units across all therapeutic areas have evaluated and expressed interest in the Theranos infrastructure

- Theranos and GSK have a fully executed MSA

- Integrated architecture of Theranos infrastructure requires adoption at top corporate levels

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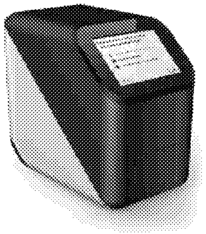
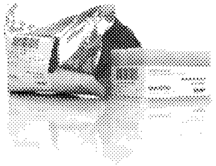
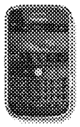
Theranos Infrastructure Technologies

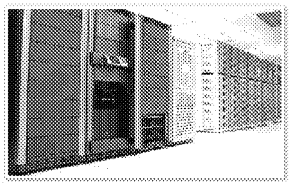
Theranos Field Systems

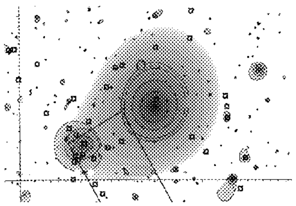

Devices

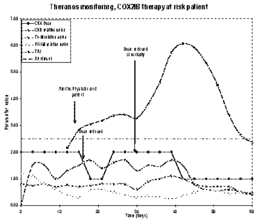

Cartridges


Mobile Applications – Ex. the Health Assistant

TheranOS – Theranos Operating System


Data Infrastructure


Models and Algorithms


Decision Support Applications – Ex. Virtual Studies

 THER-6460780

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GTS

- GTS is a fully integrated, enterprise wide health data capture (including blood testing), analysis and care delivery solution
- Accelerates clinical development timelines, improves probability of success (POS) of realizing each target product profile, and increases physician and patient adoption (increases sales)
- Comprised of Theranos Field Systems and the TheranOS
  - Integration of technologies and more frequent sampling identifies predictive signatures that have not been possible to characterize using the conventional analytical infrastructure (movie v. snapshot) to better and more rapidly characterize efficacy and safety
  - Infrastructure is self-learning and is refined with every new data point collected across any business unit
  - Provides predictive decision support tools for clinicians
  - Provides actionable, “smart” content back to patients to facilitate behavior modification
  - Data Collection, Analysis & Surveillance Infrastructure in emerging countries becomes care delivery infrastructure

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Contents

- Background
- GTS ROI
- GTS Deliverables
- GTS in Biologicals

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Economic Impact for GSK

- Accelerate Clinical Development/Trial Timelines

- Improve Probability of Success of Realizing Target Product Profiles

- Increase Physician and Patient Adoption – Increase sales

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Economic Impact for GSK

- Accelerate Clinical Development/Trial Timelines
  - Elimination of Logistical constraints (shipping samples, analyzing data, bringing patients into clinics, recruiting patients without knowing their response profiles, etc.) and
  - Faster, more integrated studies (adaptive trials and decision making)

cumulatively reduce development timelines by (~3) years to facilitate earlier filings.

- Theranos' large pharmaceutical clients have valued the fully loaded cost of each day gained in time to market at $1M/day

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Economic Impact for GSK

- Improve Probability of Success of Realizing Target Product Profiles
  - 5x improvements in probability of success for each asset
  - Salvage assets and improve labels (more first line therapies)
  - Realizing the improvement in attrition rate across the entire portfolio versus just one compound continually reduces the fully loaded cost of R&D
- 5x improvement in probability of success correlates with greater than 10% ROI on the total investment into a compound, averaging greater than $200M/asset

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Economic Impact for GSK

- Increase Physician and Patient Adoption
  - Evidence based guidelines for starting/stopping/re-starting therapies to increase physician comfort with prescribing
  - Rapid publications for expanded use – new indications and amelioration of safety concerns
  - Improved care delivery through individualized feedback tools and better access to medicines through Theranos' decentralized testing infrastructures (in pharmacies, through health ministries, etc.)
- Increase sales by several multiples over current adoption/projections

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Return on Investment

- The value of GTS lies in the fact that it is a fully integrated solution for data capture, integration, analysis, (and therapeutic delivery) across business units.

- The integrated solution provides compounding ROI over any particular business unit or drug-specific component.

- The key to significant ROI on GTS is programmatic deployment, which yields short term cost savings against the initial customization investment in addition to longer term ROI measured in terms of time saved and improved POS of realizing the target product profile for each asset.

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Immediate ROI: Executing on Healthcare Diversification Strategy

GTS is the vehicle for execution of GSK's strategic priorities and realization of the associated impact to earnings and growth

- Accelerated timelines … simplifying GSK's operating model
- Improved POS … delivering more products of value
- Increased adoption … growing a diversified global business

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Contents

- Background
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- GTS Deliverables
- GTS in Biologicals

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Theranos is the only company with full integration between sample analysis and analytical capabilities

GTS integrates patient sample analysis with sophisticated analytical capabilities to increase R&D ROI.

Capability	Clinical Trial Simulator	Physiological Modeler #1	Physiological Modeler #2	Central Lab	CRO	theranos redefining healthcare
Patient recruitment					✓	✓
Investigator/site mgmt					✓	✓
Sample handling				✓	✓	✓
Sample analysis				✓	✓	✓
Data management	✓	✓	✓		✓	✓
Basic analytical package • PK/PD modeling • Clinical trial simulation	✓	✓	✓			✓
Physiological model		✓	✓			✓
Dynamic learning models and real-time data acquisition						✓
Clinical study report					✓	✓

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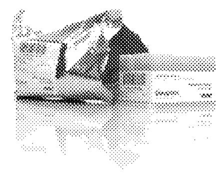
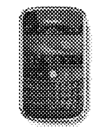
Theranos Field Systems

Devices



Cartridges



Mobile Applications –
Ex. the Health Assistant

- Measure whole blood analytes from a finger stick in real-time at any desired point of care (home, clinic, or mobile units)
- Simultaneously collect behavioral and lifestyle information through intuitive graphical touch screen interface
- Data from each device automatically and securely transmitted to TheranOS in real-time through cellular network
- Actionable information sent back to devices and applications (i.e., the Health Assistant, Virtual Studies Application)
- Point-of-care analysis of fresh whole blood eliminates conventional testing infrastructure issues, such as:
  - Analyte decay rates
  - Volumes of blood and frequency of blood draws
    - Decreases sample volume by 98%
    - Sampling schemes no longer restricted
  - Cost and logistics of sample shipments

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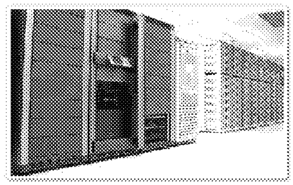
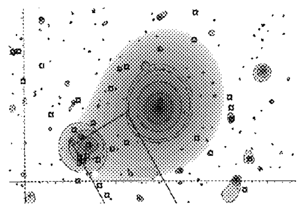
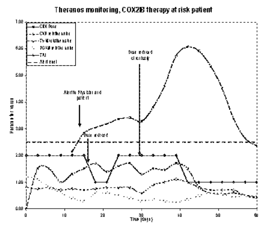
TheranOS



Data infrastructure



Models and Algorithms



Applications – Virtual Study

- Data Infrastructure
  - Automatically imports data from any desired source.
  - Translates it into one standardized format.
  - Self-learning data engine
- Models
  - Dynamically models the integrated data sets in real-time
  - Fully integrated and inter-connected physiological, statistical, and epidemiological system
  - Characterize each compound's mechanism-of-action.
  - Characterize all pathophysiologies associated with realizing each compound's target product profile
- Customized Applications
  - Clinical trials simulation
  - Adaptive trials management, in compliance with existing regulatory guidelines
  - Accessed through secure online web portal

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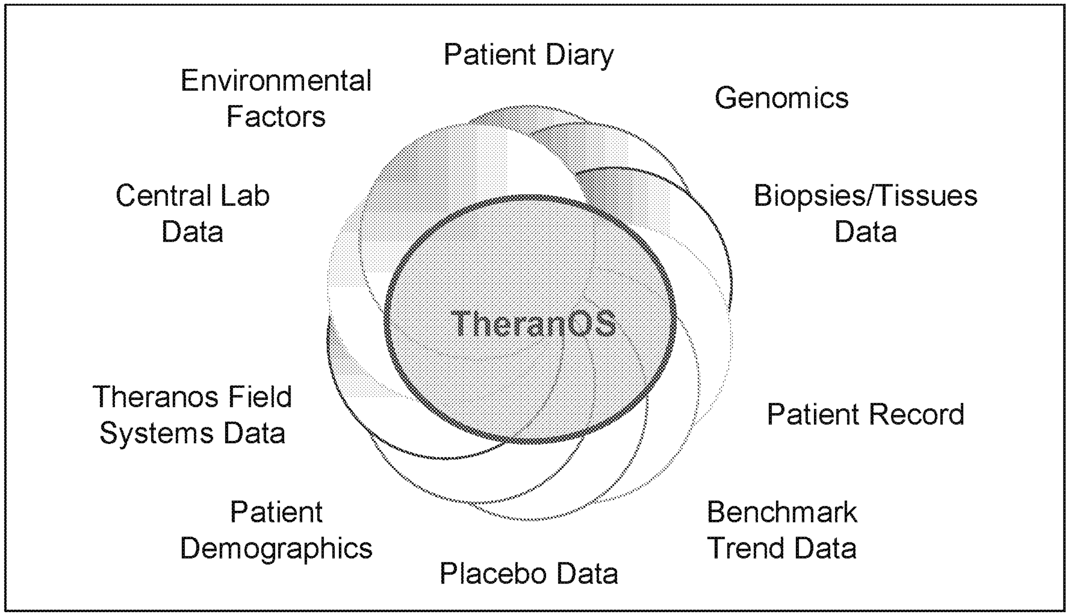
TheranOS: Proprietary Data Integration, Translation

- Proprietary import tool on web portal allows for automatic importation and standardization of data from all clinical databases.
- All data is automatically integrated with Theranos Field Systems data, centralized, and passed through predictive models.



TheranOS Data Infrastructure

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GTS Architecture Drives Deployment Plan

- A cornerstone of GTS' architecture is the inflammation engine.
- The central role of inflammation in the disease process and tissue damage/repair, allows one to apply the GTS infrastructure across various therapeutic areas and business units.
- Deployment of a customized inflammation platform provides the ability to rapidly integrate data from different pathophysiological states for predicting and establishing novel therapeutic indications.
  - GTS engine learns from every new data point and models become increasingly predictive -compounding predictive power
- Drug-specific models and cartridges are built on GTS' pathway architecture to conform to existing business unit structure.
  - TheranOS allows for data integration & exploitation across a broad range of existing data capture tools.

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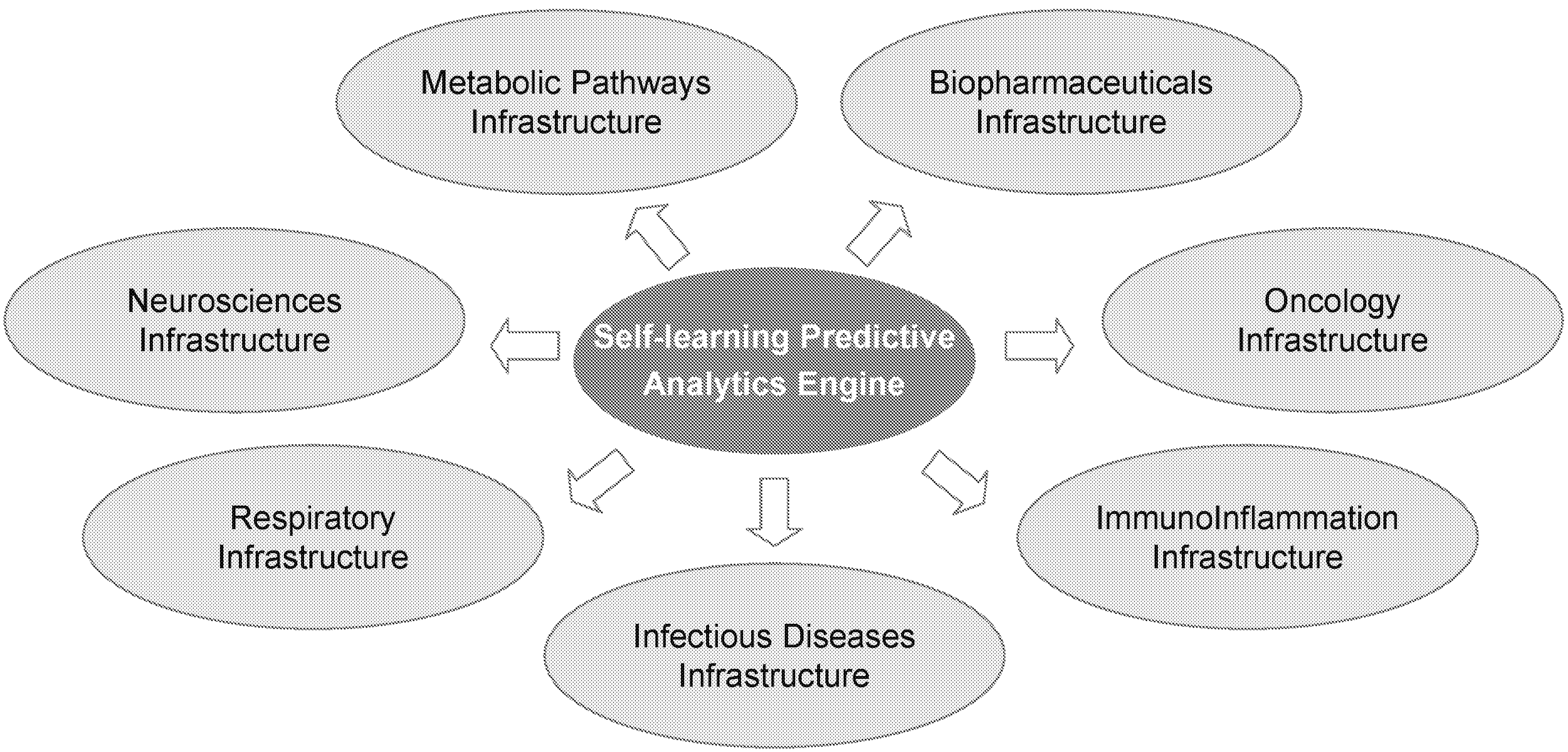
Rapid Customization of GTS: Therapeutic Area Infrastructures



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Decision support applications:

TheranOS Software for each Therapeutic Area:

- Probability Mapping Application
- Health Assistant
- GTS Assistant
- Adaptive Studies
- Ontologies
- Predictive Signatures
- Biomarker Identification Application (BIA)
- Virtual Study Application
- Others

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Data Collection Library & Care Delivery Tools:

For each therapeutic area:

- Cartridge tests – libraries of ~250 tests per disease area
- Device touch-screen software applications and embedded sensors – blood pressure, weight, others
- Mobile phone applications

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Rolling infrastructure set-up

Data Infrastructure

Inflammation Engine

7 Therapeutic Area Infrastructures

Customization and activation of base GSK data infrastructure and learning engines followed by rolling set up of 7 therapeutic area infrastructures

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Rolling infrastructure set-up

Data Infrastructure

Inflammation Engine

7 Therapeutic Area Infrastructures

- Biologicals
- Prescriptions
- Consumer

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Rolling infrastructure set-up

- Biologicals: Influenza (vaccine) → Oncology → Others
- Prescriptions: Unprecedented Early Development Compounds, REMS, LpPLA-2 → Early Development, Phase III, Phase IV & Post marketing studies
- Consumer: Weight loss (alli) → Smoking Cessation → Others

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Deployment of GTS

- Customization, Installation, and License of enterprise infrastructure
- Deployment of consumables for studies
- License expansion – Deployment of additional drug-specific models/consumables

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Contents

- Background
- GTS ROI
- GTS Deliverables
- GTS in Biologicals

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Rapid Validating Efficacy of Existing Vaccines Against Drifted Strains of Influenza Virus

- Theranos characterized relationship between dose, clinical efficacy, and antibody titers to influenza strains on its validated point-of-care systems.
- Assays identify functional, strain-specific antibodies from a finger-stick of fresh whole blood.
- Once deployed in a clinical study, patients could be immediately challenged with the actual virus and followed for 2+ weeks to assess whether the existing vaccine is efficacious.
- If not, the same infrastructure could be used to rapidly assess optimal dose and efficacy of a new vaccine.

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Influenza Surveillance Infrastructure

Real-time development and deployment of antibody, cytokine, and efficacy/safety marker measurements from finger-stick of blood /nasal swab run on point-of-care device

- Characterize velocity of antibody decay
- Accelerate development of new vaccines to mutations
- Quantitatively characterize efficacy and safety profiles to ameliorate concerns and differentiate GSK vaccines
- Guide optimal administration of vaccines
- Provide real-time measurement of efficacy and immunity

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Influenza Surveillance Infrastructure

Modeling and simulation of efficacy and safety dynamics and projected spread and mutation of the virus

- In-silico comparative effectiveness studies to optimally power head-head studies with antibody/efficacy cartridges
- Virtual studies to rapidly optimize dose and minimize safety issues
- Rapidly power (adaptive) studies
- Detect any mutation of the H1N1 virus as it emerges.
- Project spread of disease and mutations

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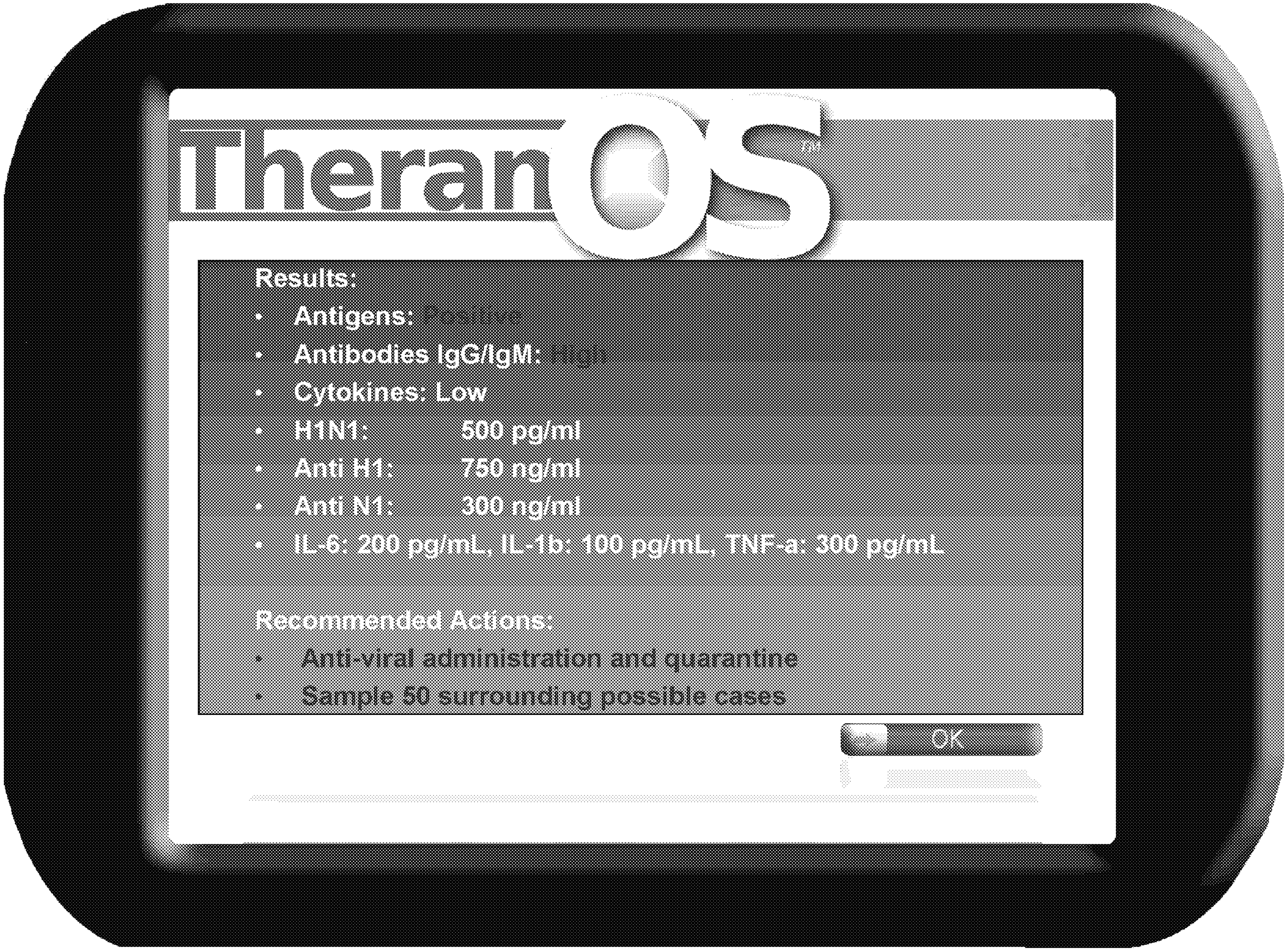

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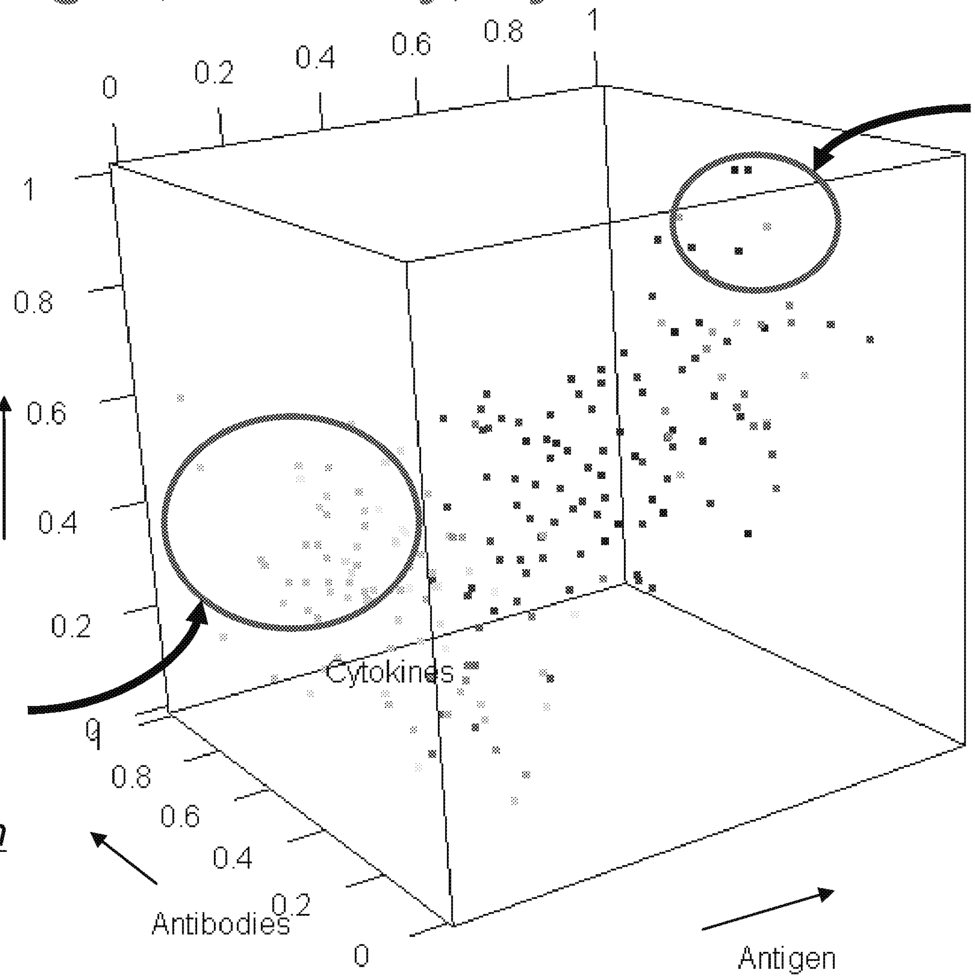
Recommended Actions Depend on Levels of Antigen, Antibody, Cytokine and Other Markers

Combination of low Ag, low cytokine, and high Ab suggests *prophylactic anti-viral administration and quarantine*



Combination of high Ag, high cytokine, and low Ab suggests *hospitalizations*

Colors classify patient "groups" for triaging optimal intervention

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THS Modeling Platform Capabilities

1. Predicts spread of an infectious pathogen in a heterogeneous human population.
2. Reflects the impact of regional demographics and patient risk factors.
3. Enables evaluation of healthcare mitigation policies, for example:
   - Surveillance/testing strategies
   - Hospitalization, home isolation, and quarantine policies
   - Prophylactic vaccination and anti-viral treatment policies
   - School and workplace closures; other social distancing measures

   Enables cost assessment and evaluation of quality adjusted life years (QALY) saved by comparing alternative mitigation approaches.
4. Is fully integrated with real-time data acquisition, enabling model updates based on the latest data acquired from multiple sources
5. Includes automated, frequent model updates.
   - Leads to more accurate projections for spread.
   - Allows health agencies to rapidly adapt to changing conditions.

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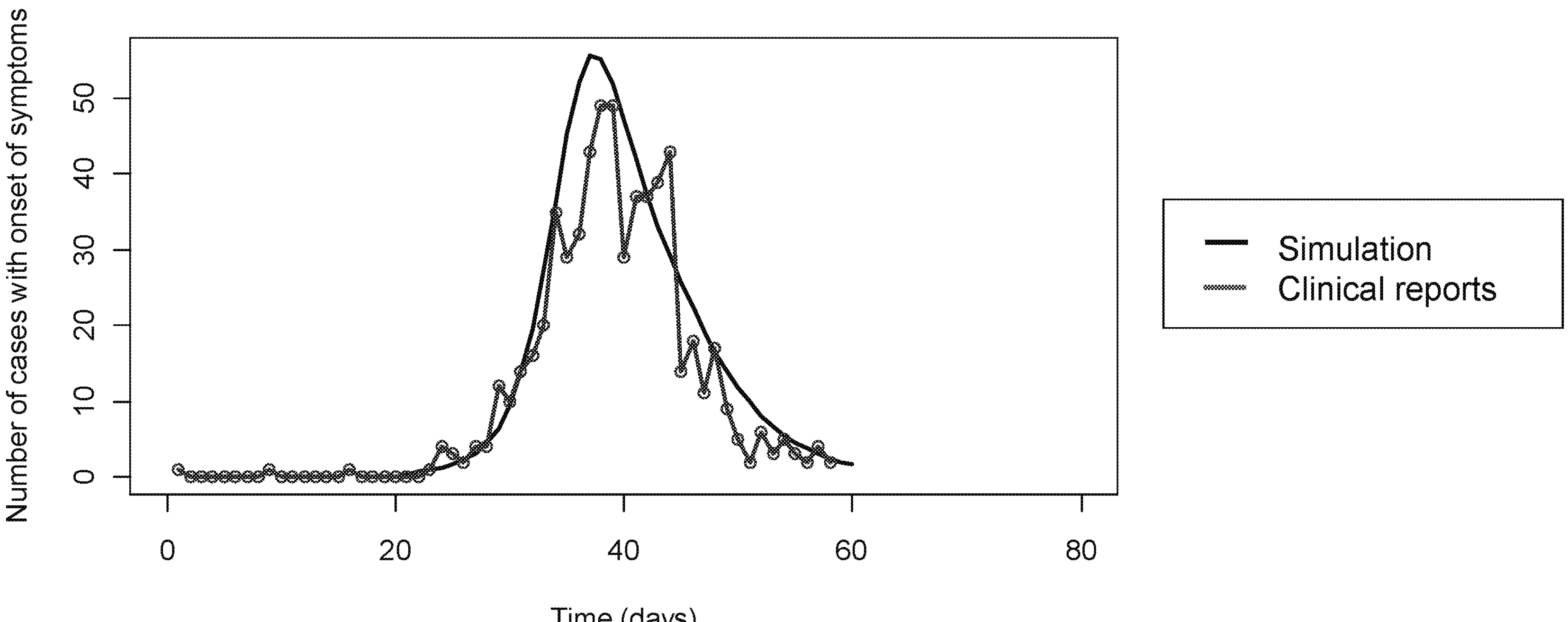
THS Model Accurately Reproduces Spread of La Gloria Outbreak

- All models are validated by reproducing historical data



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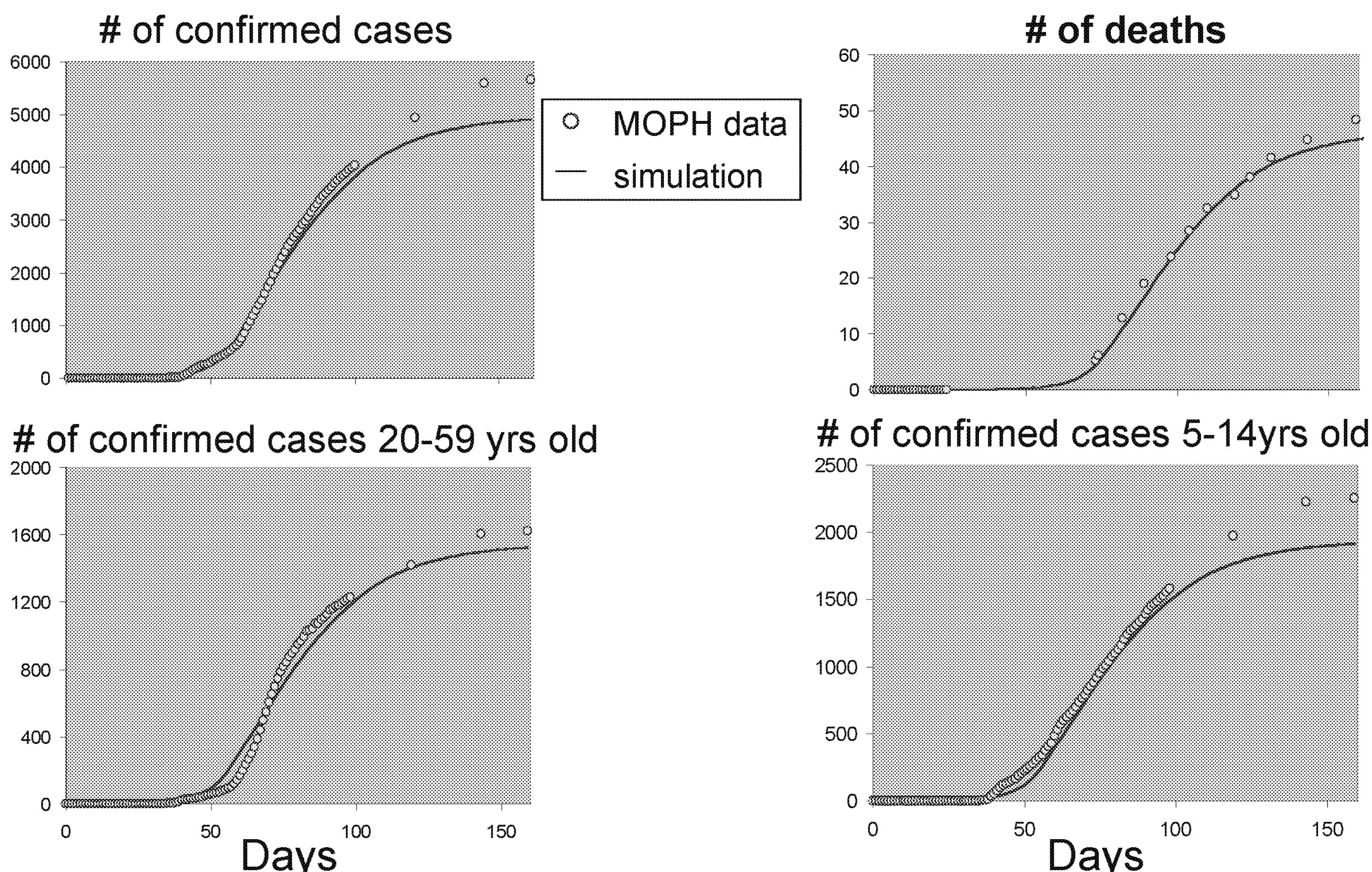
Model Reproduces Bangkok Publicly Reported H1N1 Data Including Deaths and Age-Dependence

Total cases ~20,000; reported cases significantly less.



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Selected Oncology Applications

- Rapid expansion of use through predictive visibility (models) and early reads (cartridges) on effiacacy and safety in new indications
  - MAGE-3 expansion
- Virtual and rapid head-head studies for comparative differentiation
  - Cervarix differentiation – characterization of velocity of antibody decay and need for re-boost
- Combination tests for low cost, real-time identification of antigen levels/presence of genetic signature from finger-stick of fresh whole blood run on point-of-care device in pharmacies, physician's offices, and other remote locations
  - MAGE-3 "responder" identification

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Deployment of GTS

- Decision support applications provide compounding predictive power
  - Inflammation/immunology/humeral response models form foundation of data analytics engine
  - Data analytics engine facilitates data integration and connectivity between disease-specific infrastructures:
    - Viral & Allergy Vaccines
    - Bacterial Vaccines
    - Emerging Diseases & HIV
    - Cancer Vaccines

- Data collection, analytics and surveillance infrastructure faciillitates Care Delivery in emerging countries through placement of devices in remote locations

Excerpts from GSK Metabolic Study Report

Nelson Rhodes, Director GSK Metabolic Biomarker Laboratory
Surekha Gangakhedkar, Theranos Assay Systems Lead

Background information:
The Theranos system was evaluated at GSK to profile active GLP-1 and C-peptide values and these data were compare to "gold standard" ELISAs using frozen human plasma from study XXXXXXX. The key project objectives (found in the attached statement of work) were:

- To assess the performance of the Theranos System in measuring a multiplex for GLP-1 and c-peptide values (the "Cartridge Analytes") as compares to the current gold standard ELISAs (which are not multiplexed).
  - Specifically, the study will assess Theranos' capabilities to detect points that the reference assays failed to accurately detect by running samples with C-peptide values in a standard range (ng/mL) and GLP-1 values between 0-3.2 pM
- To assess the functionality, specificity, reproducibility, accuracy, and precision of the Theranos System.
- Assess the Theranos data reporting and transfer functions

Thirty plasma samples (assayed in duplicate) were chosen based on historical GSK data for total GLP-1 levels from subjects given a mixed meal and two finger prick blood draws were performed. Five Theranos machines were used with active GLP-1 and C-peptide cartridges that required 20µL of plasma. MesoScale Discovery's (MSD) active and total GLP-1, Linco (Millipore) active GLP-1, and Linco (Millipore) C-peptide ELISAs were run as comparator assays.

GSK Metabolic Biomarker Lab comments:

- Data show good correlation
  - $r^2 = 0.90$ for GLP-1 (MSD vs. Theranos)
  - $r^2 = 0.96$ for C-peptide (Linco vs. Theranos)
- Inter-instrument precision (RLU average %CV = 11)
- Machines worked well
- Touch-screen interface was easy to use
- Cartridges were pretty straight forward (easy to handle and load)
- Assays took approximately 1 hour and 15 minutes per cartridge

Overall conclusions:
- The Theranos system eliminates the need for a lab and provided quality data
- The Metabolic Biomarker Lab has a favorable impression of the technology/system and recommends GSK clinical groups to work with Theranos

Data:

Study design

- Human subjects
- Food “challenge”
- Measure GLP-1 and C-Peptide multiplex over 5 time points
  - – Linco Assay
  - – MSD Assay
  - – Theranos Assay

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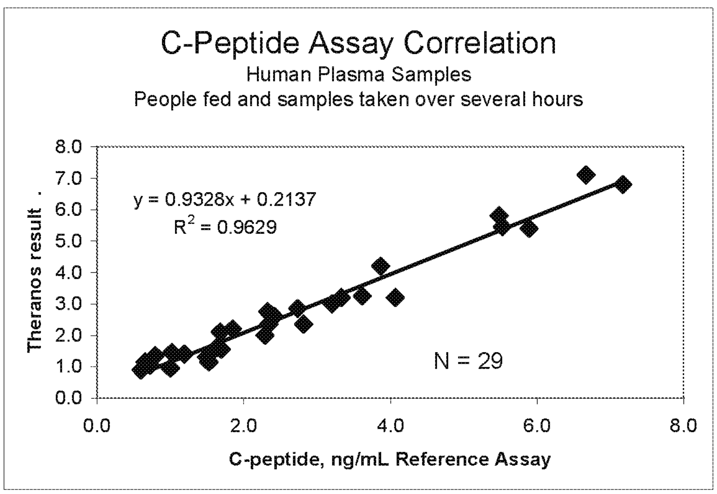
C-Peptide Assay

Averaged results



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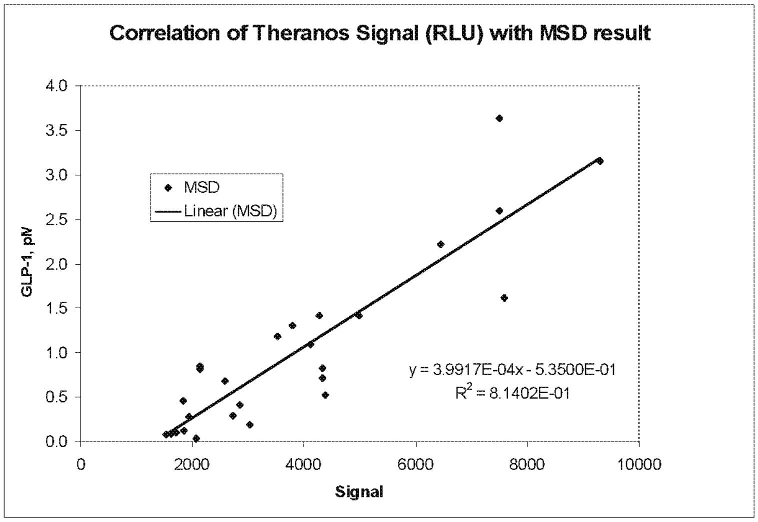
Calibration to GSK matrix



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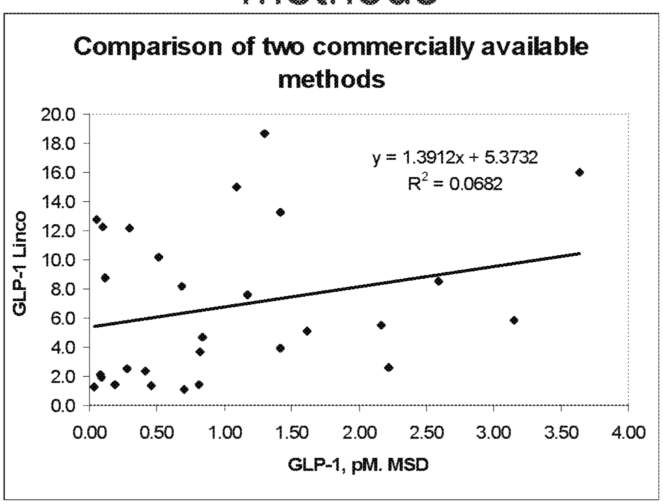
Lack of correlation of predicate methods



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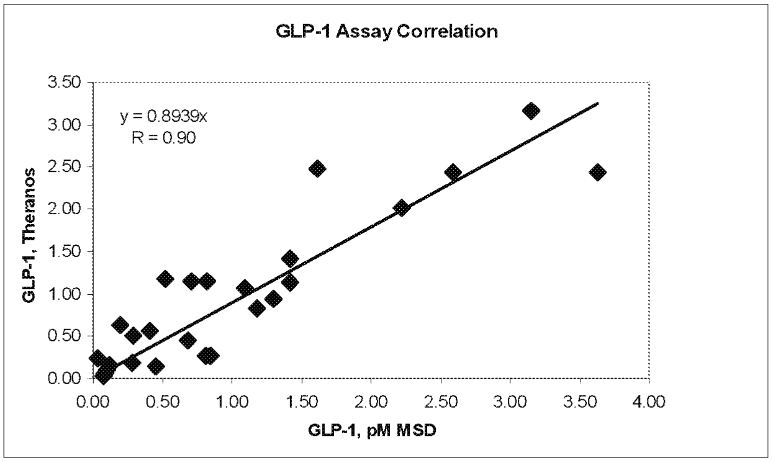
Assay correlation



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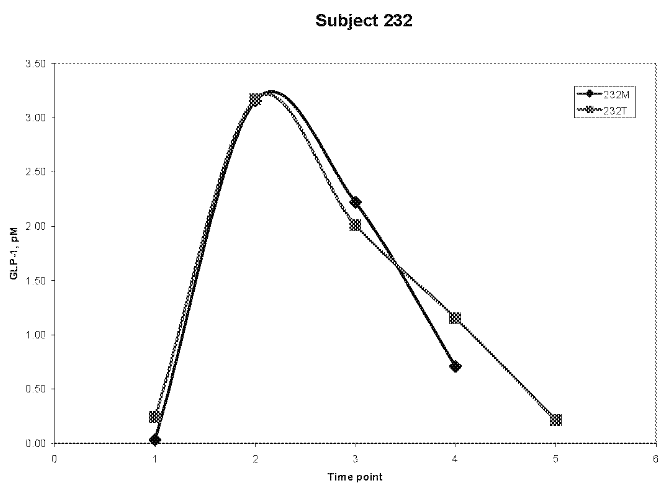
Subject 232



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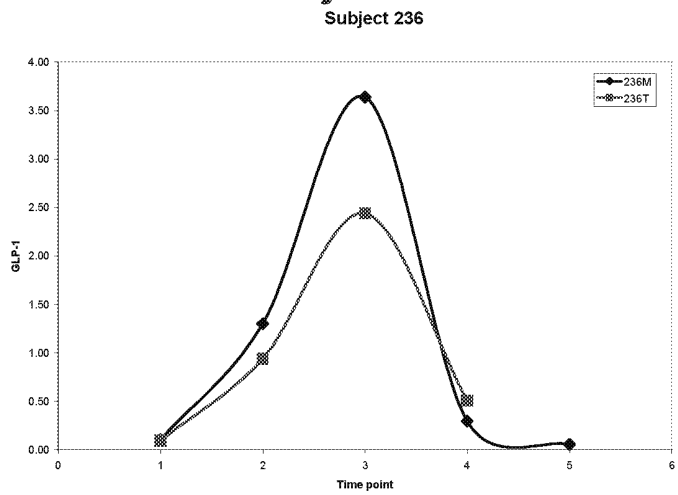
Subject 236



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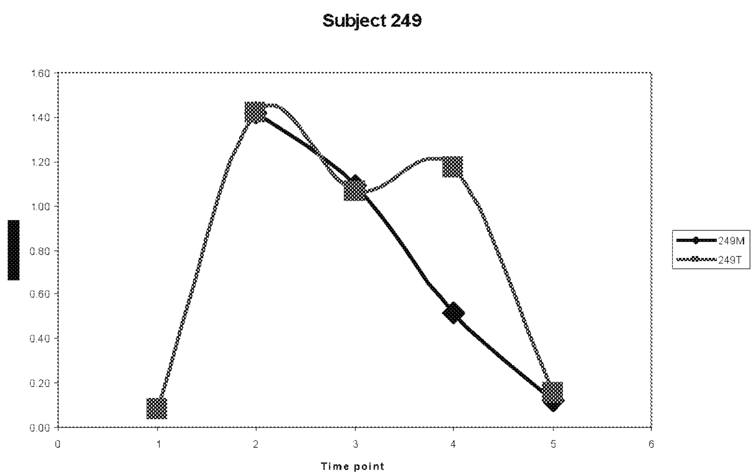
Subject 249



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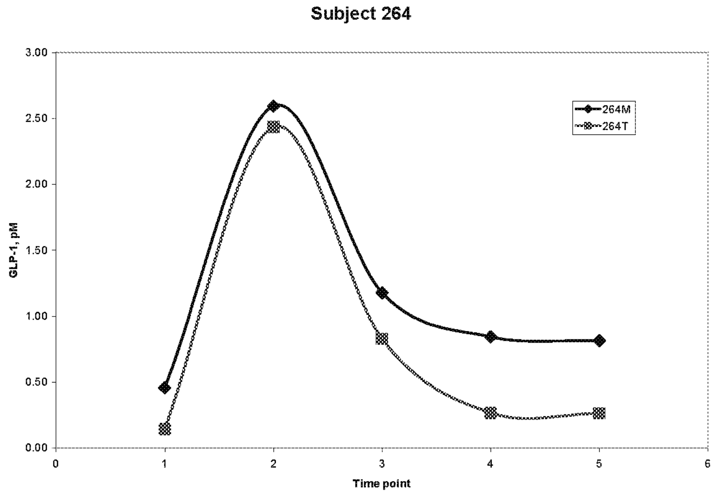
Subject 264



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Summary Statistics GLP-1 Comparison

- Theranos LOD = 0.17 pM
- Dynamic range measured: 0-3.2 pM
- Mean = 0.9 pM (Th), 1.0 (MSD)

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TPS Case Study: Client ROI

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Virtual Study Application

TheranOS Virtual Study Application enables more efficient clinical study design, conduct, and analysis through in-silico:

1. Comparison of alternative clinical study designs
2. Exploration of drug effects on multiple physiologic outputs
3. Examination of patient response variance in order to power the clinical study
4. Optimization of dose regimens
5. Examination of the magnitude and variance of side effects

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Virtual Study Application

TheranOS Virtual Study Application enables more efficient clinical study design, conduct, and analysis through in-silico:

6. Identification and selection of sub-populations having different physiologic responses
7. Identification of predictive patterns for early reads on efficacy and safety
8. Refinement of enrollment criteria.
9. Probability analysis of likely clinical outcomes for a given design.
10. Head-head studies for comparative effectiveness
11. …

Simulations can be run before a study is designed and dynamically throughout each study.

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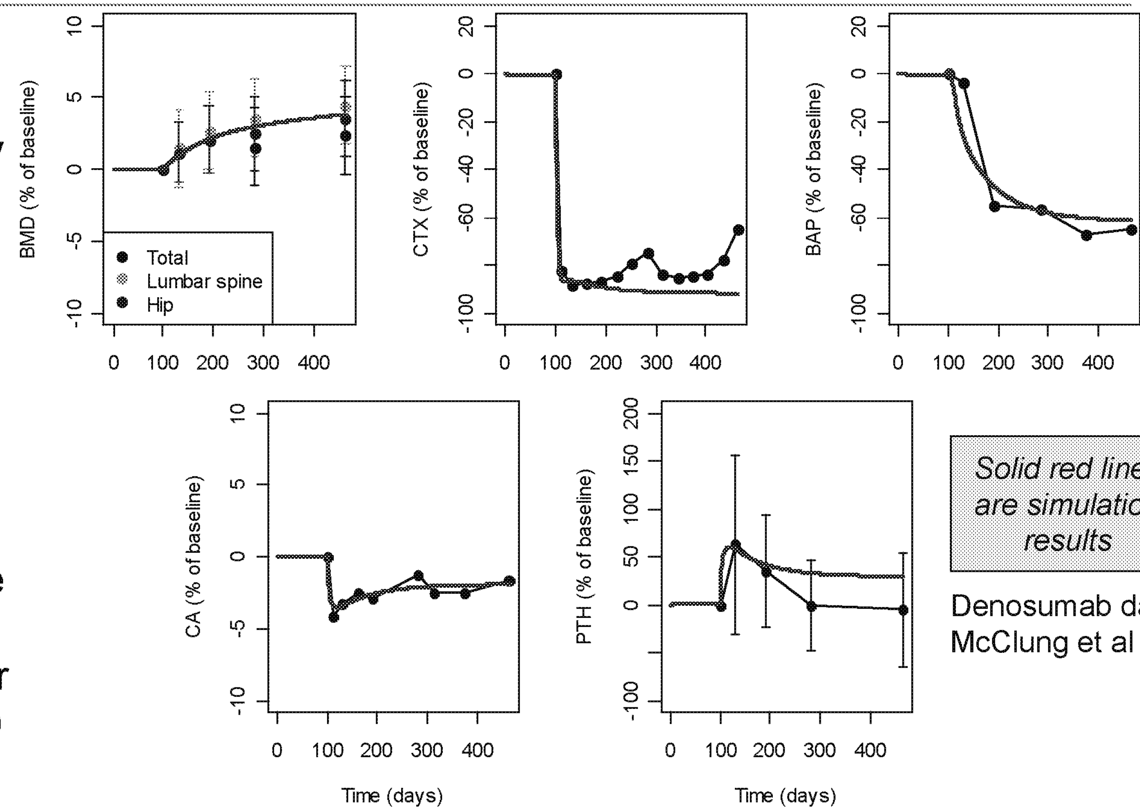
CASE STUDY M

TheranOS Comprehensive Physiological Models

Using the interconnected physiological modeling engine, simulated optimal therapy regimens for maximum efficacy and minimal adverse events for asset that acts on multiple pathways.

- 95% target inhibition reproduced key behaviors reported in the clinical study of compound
- The model predicts the efficacy profiles of the drug, even without accounting for its *mechanism of action* (MOA models built for other drugs)
- Model identified a predictive signature of BMD that is measurable ~6 months prior to physical changes in BMD
- www.theranos.com



Solid red lines are simulation results

Denosumab data: McClung et al 2006

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CASE STUDY B

Example of TPS in Compound Development for Anemia and Bone-related Disease

1. Customized TheranOS for automated data integration, analysis and real-time self-learning
   – Compounding predictive power from all Client-generated data
2. Developed and validated physiologic-based mechanistic modeling and simulation system
   – Captured effects of target inhibition by Compound treatment
   – Included target patient phenotypes based on literature and healthy patient responses to Compound
   – Optimized design, evaluation, execution of (adaptive) clinical trials for Compound
   – Led to novel biomarkers for efficacy and/or safety, enhancing patient treatment with Compound

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CASE STUDY B

Example (cont'd)

3. Virtual Study Application used to optimize Phase IIa trial design for target patient population
   - Recommended designs enhance power of trial
     - Increased probability of success
     - Provided support for regulatory reviews
   - Integrated data sets and models used by Client to run in-house simulations
   - Easy-to-use interface for in-house ownership/use of highly complex, proprietary modeling system
4. TheranOS applications integrated with Theranos Field Systems yielding compounding predictive power
   - Automated data integration, analysis, self learning and model refinement for trial design, analysis, and patient monitoring
   - Extended to include additional indications for Compound and for other compounds and their indications/target profiles

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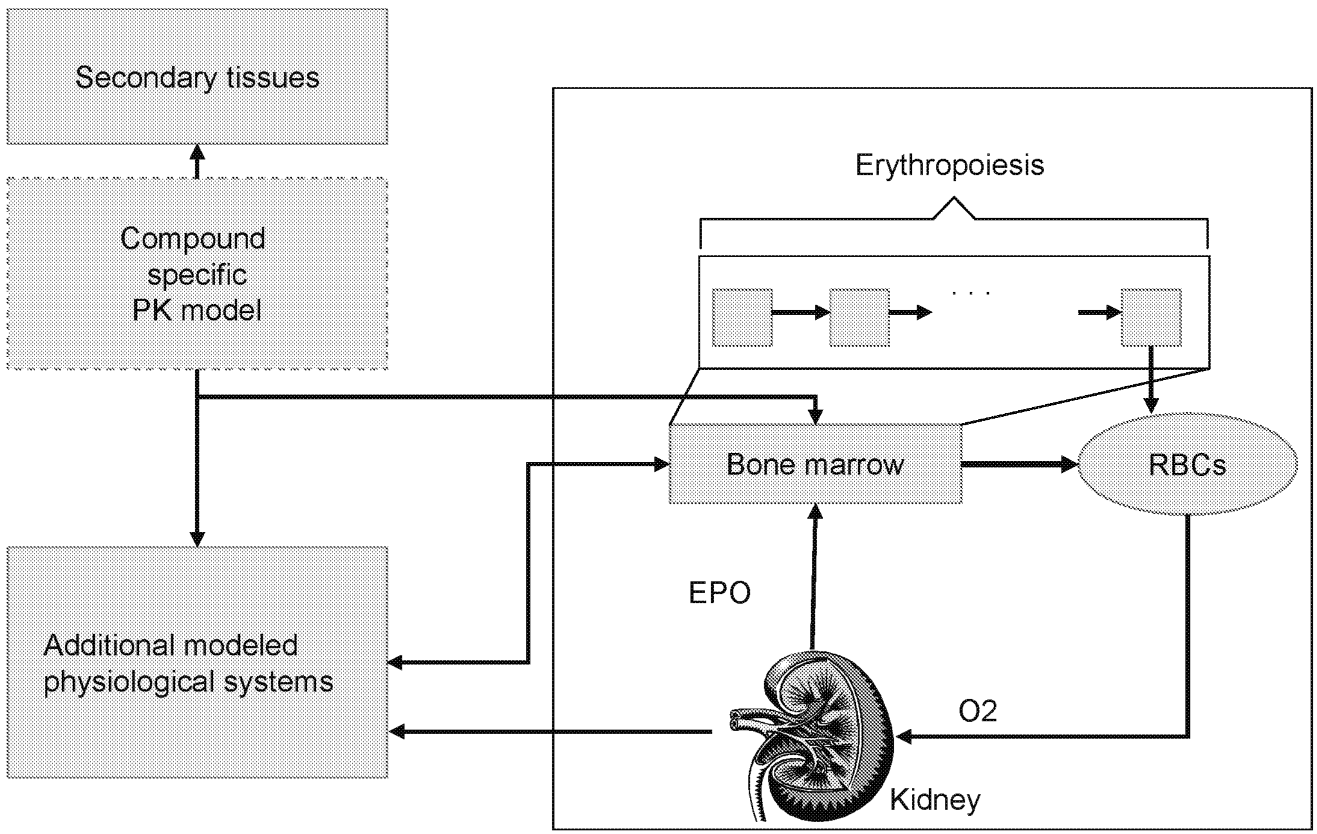
CASE STUDY B

Schematic Overview of Physiological Model



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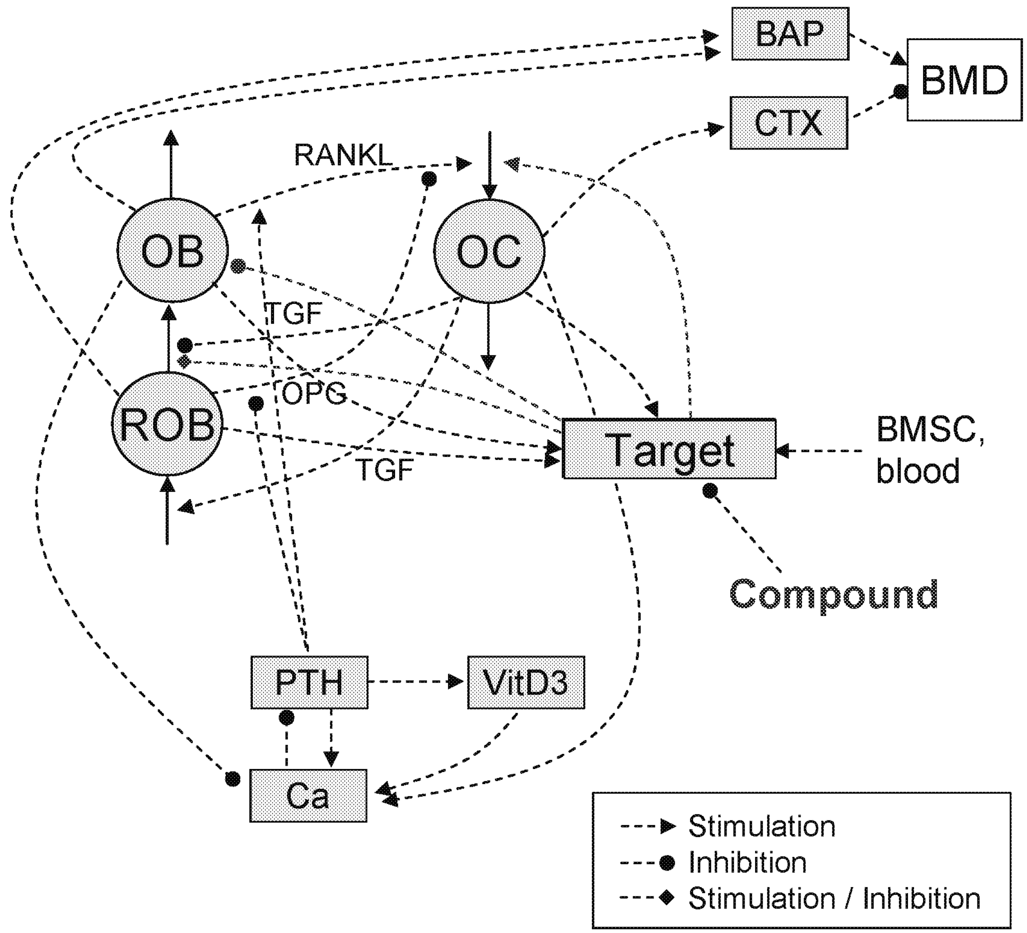
CASE STUDY B

Summary Illustration of Quantitative Model Representing the Dynamics of Bone Metabolism



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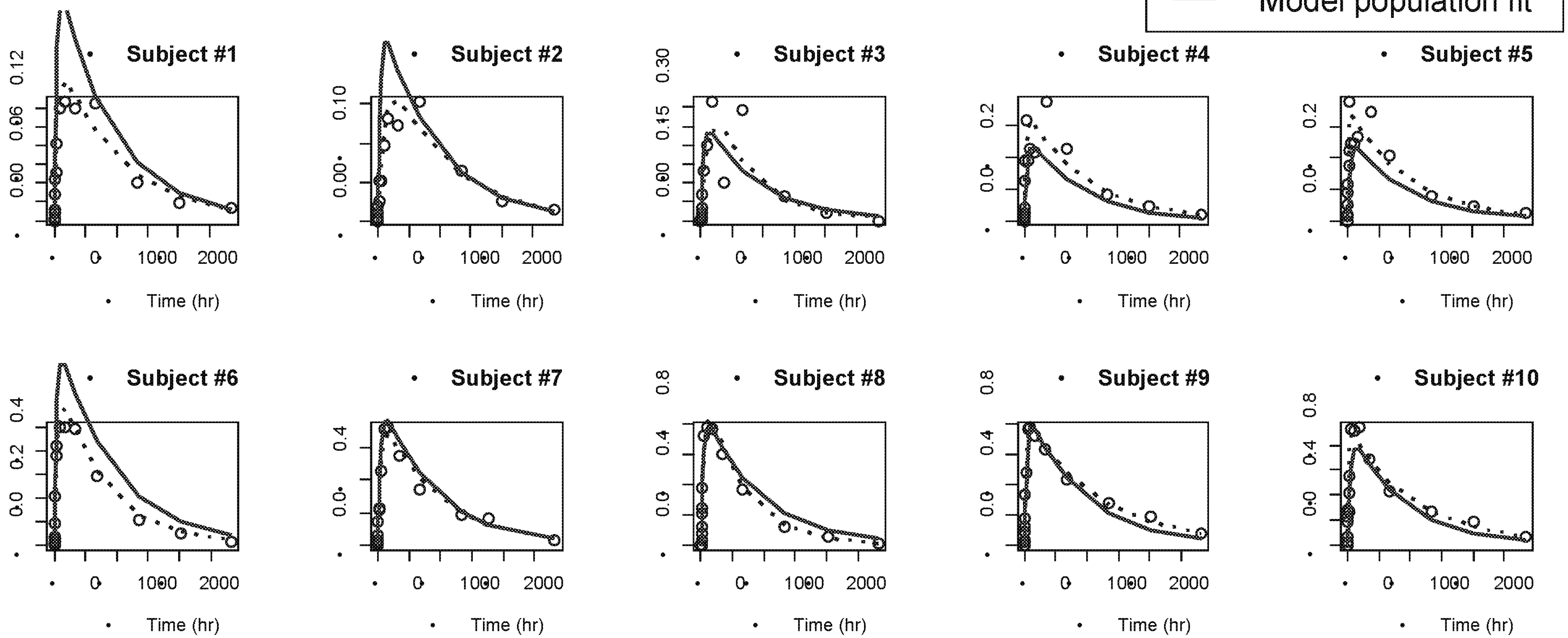
CASE STUDY B

Pop-PK Mixed-Effects Modeling for Compound SC Administration

- First-order one-compartment model was used to fit the Compound SC PK data.
- Model data accurately predicts clinical PK profiles



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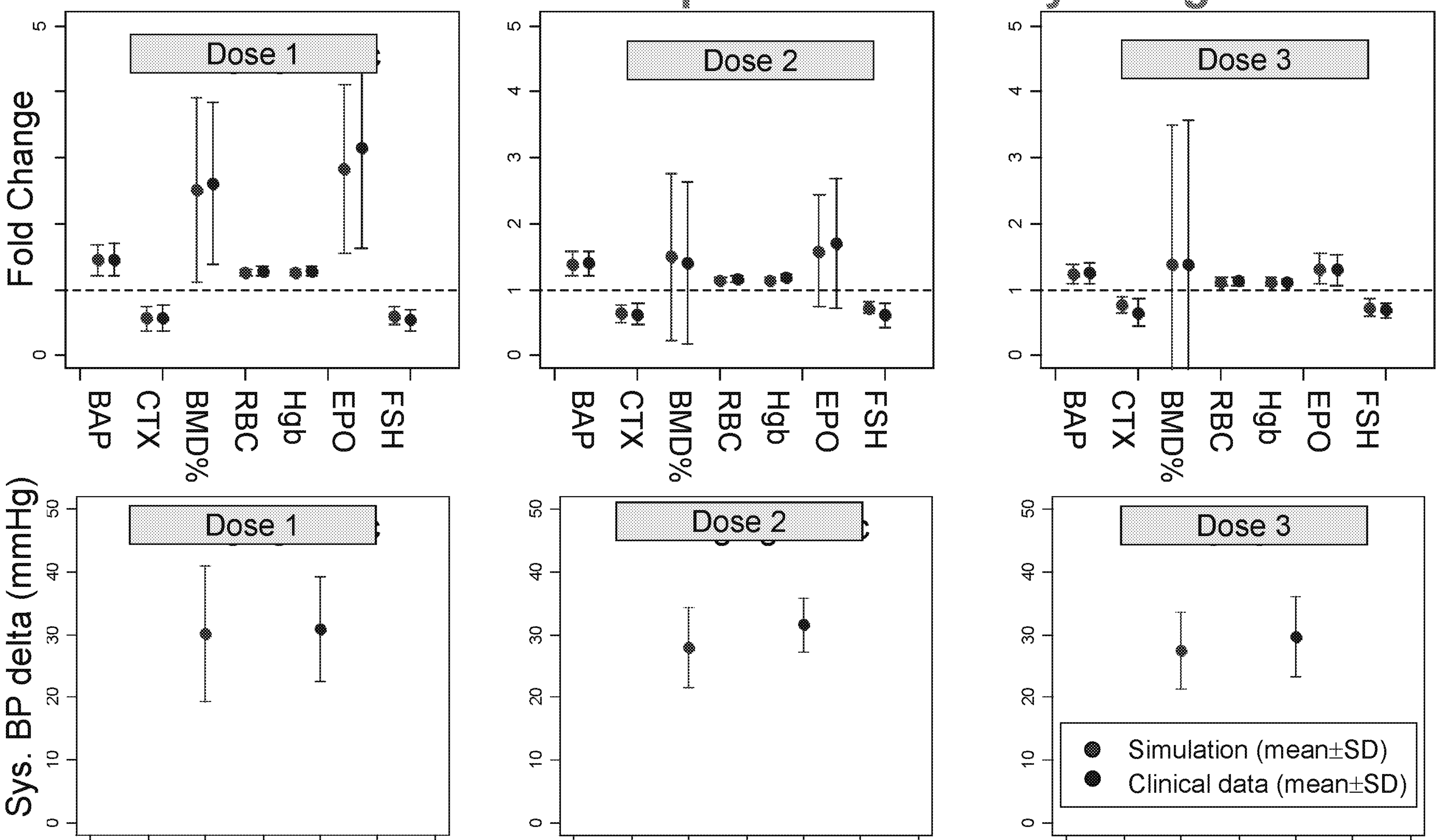
CASE STUDY B

Simulated Peak Responses Predicted High, Mid, and Low Dose Response for All Physiologies



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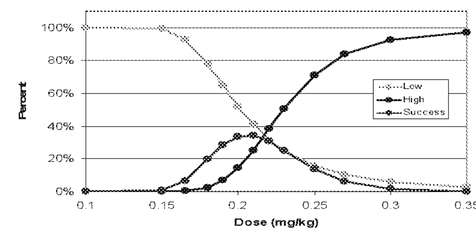
CASE STUDY B

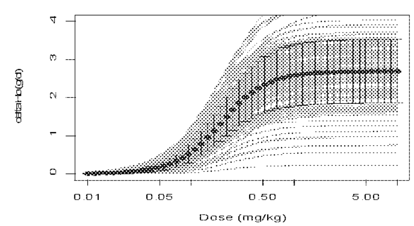
Virtual Study Application Increased Study POS

Simulations increased probability of study success by allowing users to optimize protocol and dosing titration schemes in-house prior to study initiation.

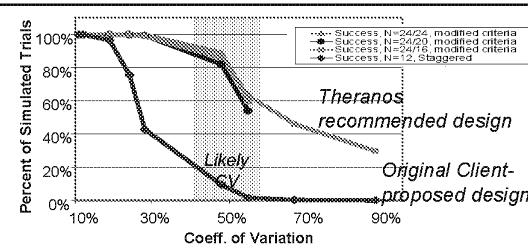
Simulation of probability of ‘successful outcome’ indicated high probability of study failure …



… due to underlying variability of responses



TPS optimized study design, dosing regimen, and titration parameters, increasing the probability of success 5x



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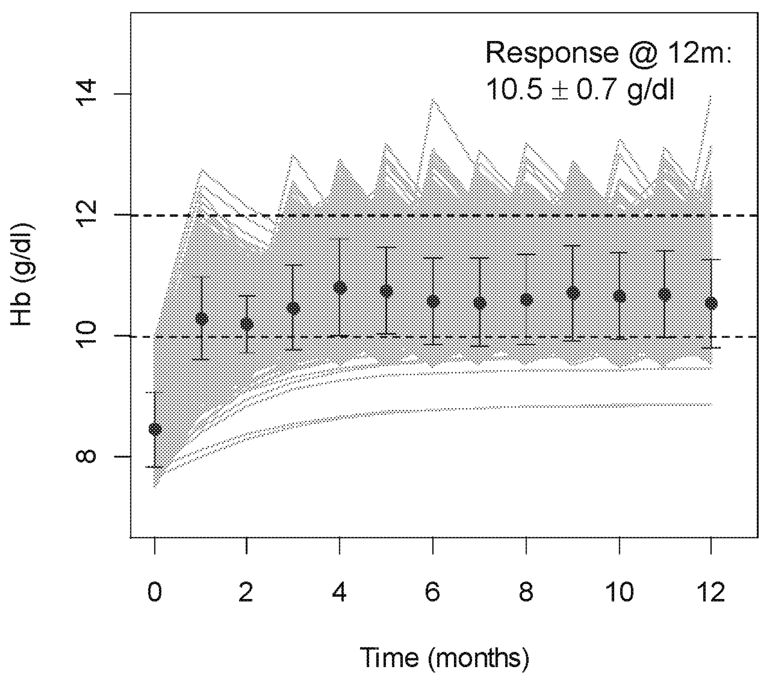
CASE STUDY B

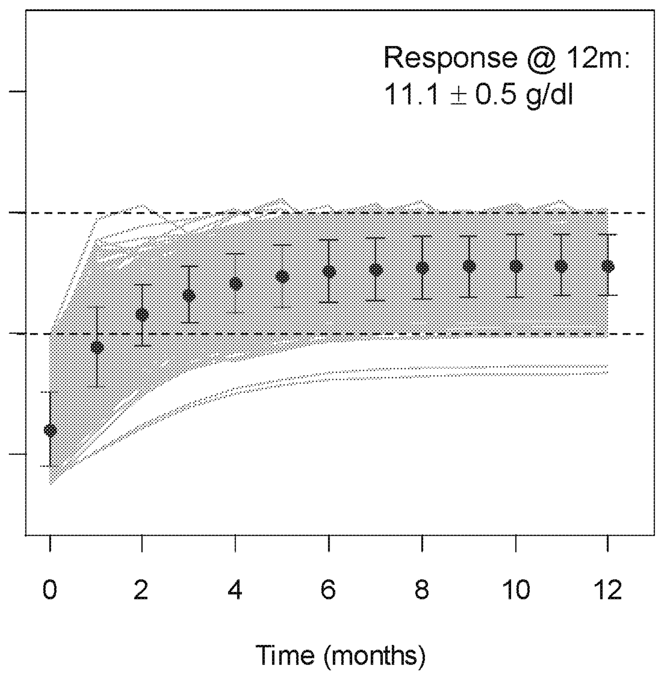
Virtual Study Application used to improve POS

New titration design resulted in lower variance, leading to fewer excursions above maximum desired response and significantly decreasing frequency of safety issues.

Initial Client Titration Design (A)



The Theranos Solution Modified Titration Design (B)



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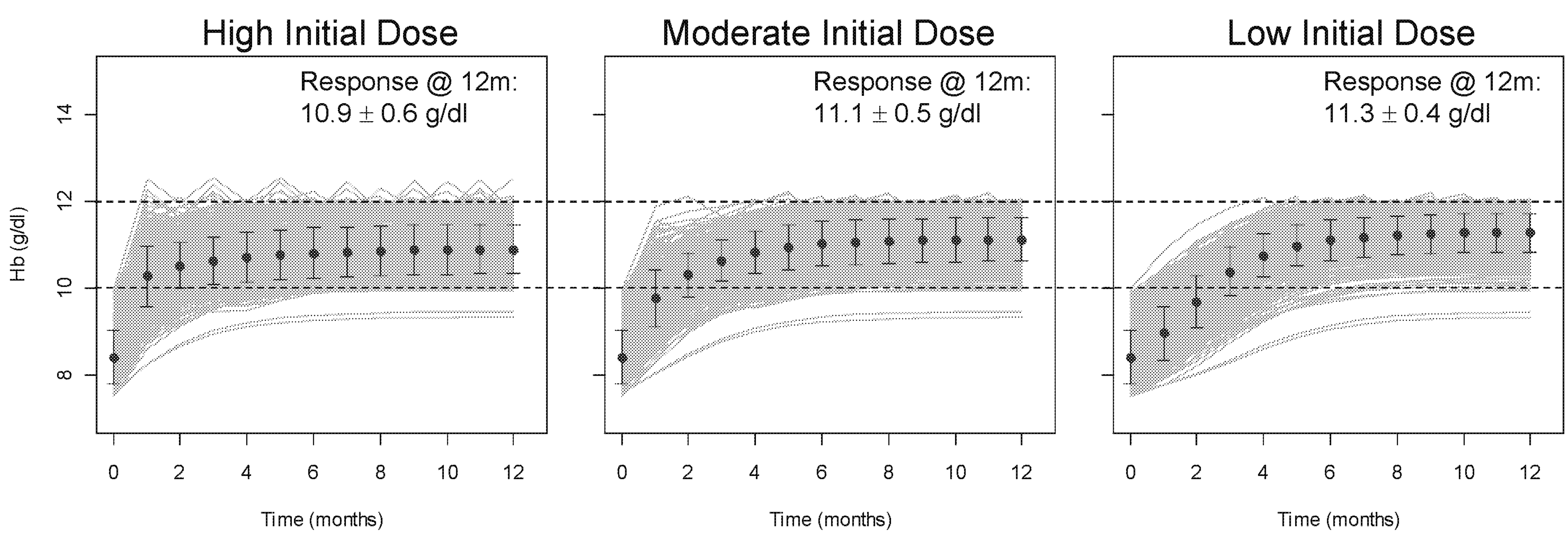
CASE STUDY B

Further Dose Titration Optimization

Further optimization of dose titration yielded even better efficacy and safety across three initial dose scenarios.



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CASE STUDY B

Safety and Efficacy Profile

Based on this safety and efficacy profile, the final design was recommended, as it:

- Significantly enhances both safety and efficacy under all conditions for heterogeneous patient populations
- Improves long-term Hgb maintenance by reducing "on-off" dosing and wide Hgb swings
- Reduces variance of Hgb response and treatment dose
- Is robust to initial dose given to the cohort

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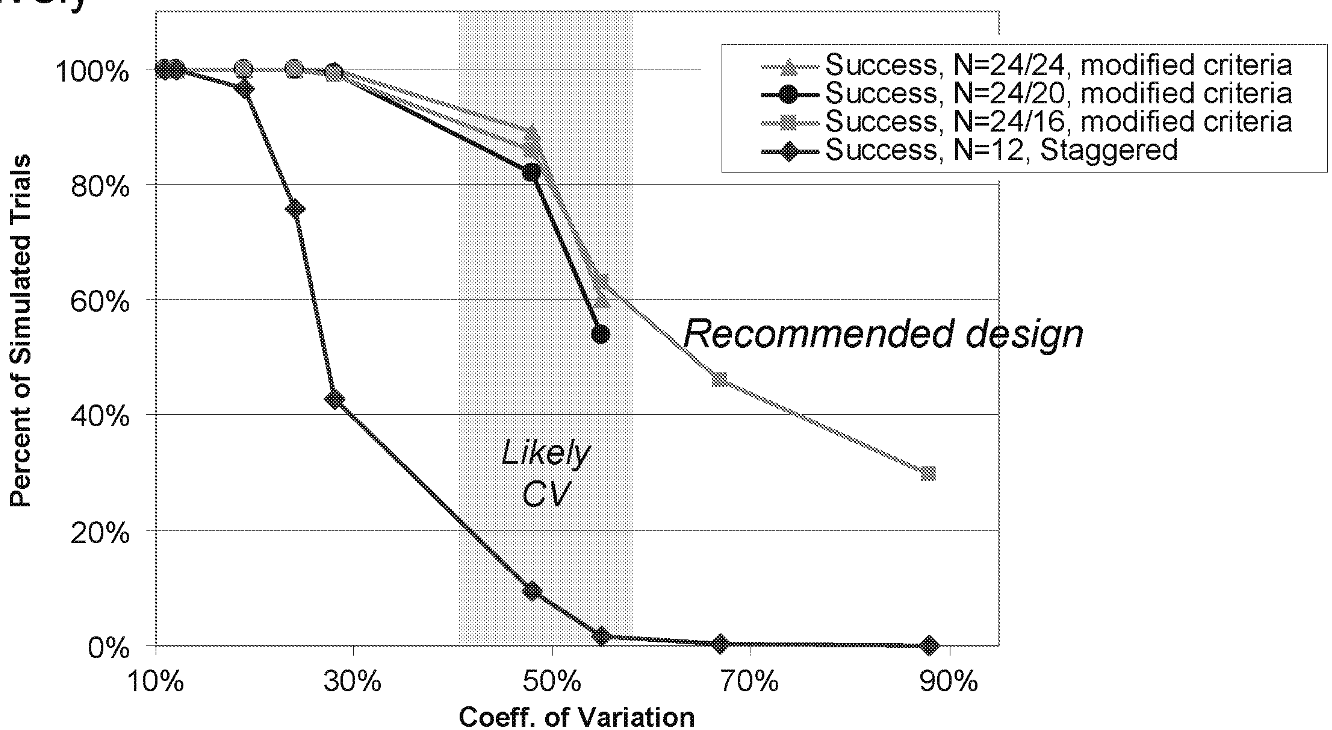
CASE STUDY B

Proposed Semi-Parallel Trial Design is Estimated to Increase the Probability of Success from ~15% to ~80%

Recommendation: semi-parallel design has good chance of success for n=24 and n=16 in initial cohort and parallel cohorts, respectively



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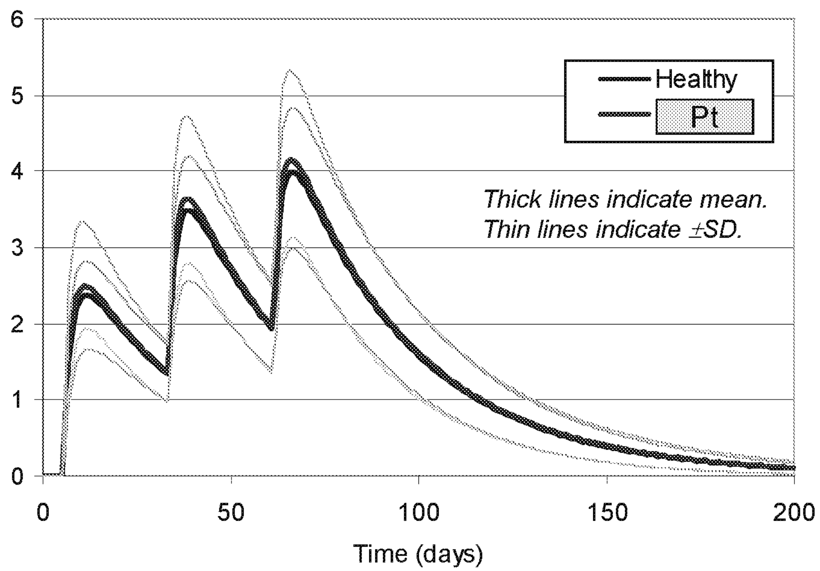
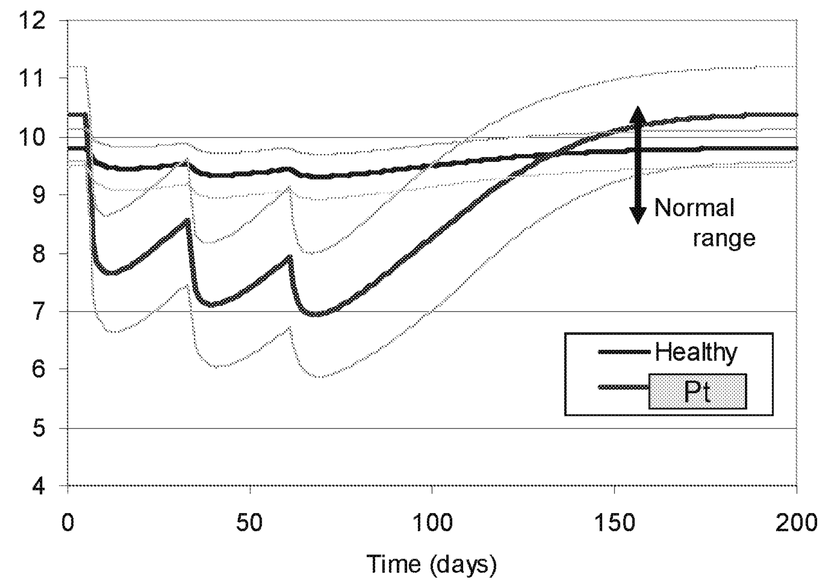
CASE STUDY B

Model Illuminates Secondary Safety Concerns

Model indicates that Compound treatment may lead to secondary safety concerns in target patients undergoing treatment, if not taken into account.





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CASE STUDY B

Secondary Safety Concerns

Model indicated that Compound treatment may lead to secondary safety concerns in target patients undergoing treatment

1. Severe hypocalcemia after intravenous administration of bisphosphonates has been observed in patients with poor mineral regulation.
2. Target patients present a particular risk due to limited endogenous mineral regulation.
3. Phase I studies with Compound in healthy patients show limited Ca effects due to normal mineral regulation in these patients.

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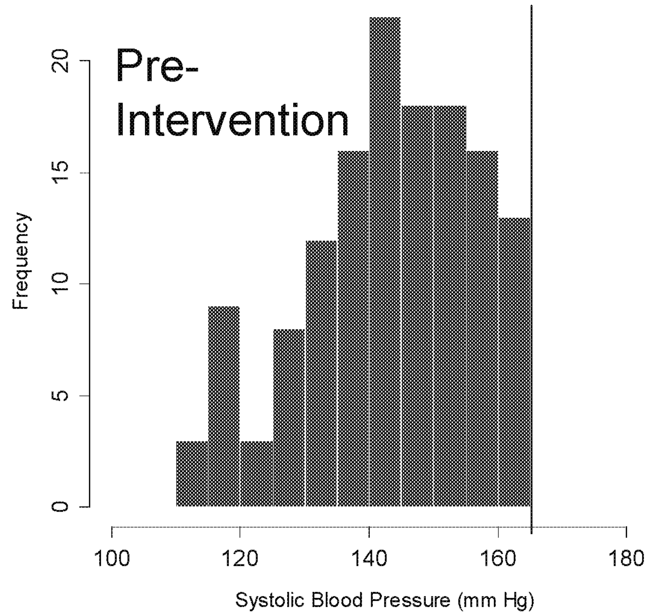
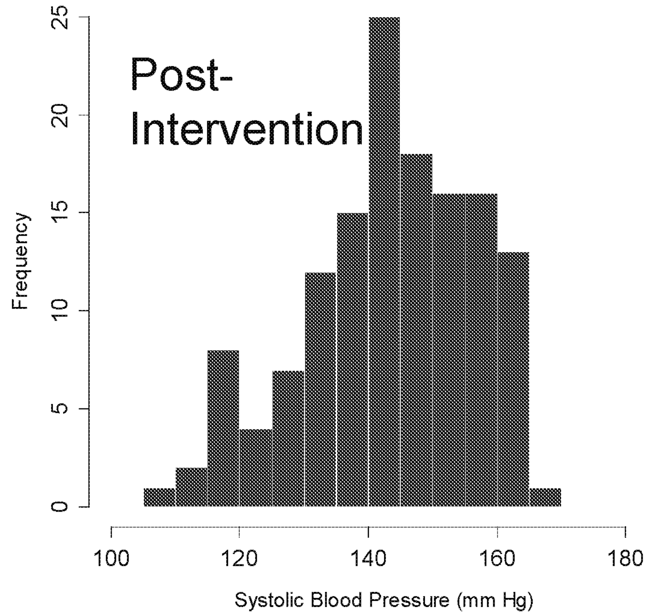
CASE STUDY B

Enhanced TheranOS Patient Cohort

After safety review, shows excellent agreement with Client data on variability in pre- and post- BP of patients.





Population mean: 150.6 mmHg — 143.2 mmHg
Population SD: 18.0 mmHg — 13.3 mmHg
[Rohrscheib et al, CJASN , 2008]

Population mean: 149.3 mmHg — 141.0 mmHg
Data from Client, Oct 27 2009

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CASE STUDY B

Summary of Dose Titration Optimization for Hgb Maintenance and BP-Related Safety Profile

Dose titration designs

	Endpoints	B3	B3a	B4	B5
All Patients	Safety profile (% population with high BP events)	8	17	8	10
	Hgb response at 1 month after last dose, (% responder patients within target Hgb range, 10-12 g/dL)	62	91	78	86
Excluding patients with baseline BP>160 mmHg	Safety profile in absence of high baseline BP patients >160 mmHg, (% population with high BP events)	0.8	6	0.8	1.6
	Hgb response at 1 month after last dose, (% responder patients within target Hgb range, 10-12 g/dL)	67	91	82	90
Implementation logistics	Information required for calculating each dose	• ΔHb since last dose • ΔHb since 1st dose • Current Hb		Additional Info • Baseline BP	Additional Info • Current BP • Max ΔBP since last dose • Max sys BP since start of trial

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CASE STUDY B

Summary of Trial Design Results and Insights Based on Modeling and Simulation

Using TheranOS model, optimized dose titration and Phase II clinical designs for target patients to meet clinical objectives, improve success probability, and accelerate development timelines

- Dose titration design predicted to improve efficacy across cohort of heterogeneous patients with improved safety profile (limits large/rapid Hgb excursion)
- Evaluated and proposed initial starting Compound dose for target patients to enhance response magnitude and rate with suitable safety profile
- Proposed semi-parallel trial design and modified success criteria predicted to increase statistical power from 15% to 80%

Selected insights based on model development included:

- Rapid hypertensive response may be due to three contributing factors: direct pharmacological effect, rise in viscosity (RBC), delayed rise in EPO (vasoconstriction).
- Identification of candidate biomarker (CTX/BAP ratio) for the prediction of BMD % change
- Delayed transient increase in EPO may be indicative of abnormal RBC/Hgb function.
- Compound treatment predicted to lead to secondary safety marker in target patients.

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ROI: Accelerating Timelines and Improving POS

Milestones **Theranos impact**

- Program cycle time
  - Critical path studies ✓
  - Individual study cycle time
    - Design and planning
      - Protocol synopsis to approval ✓
    - Startup and initiation
      - Protocol approval to FPFV ✓
    - Conduct and closeout
      - FPFV to LPLV ✓
      - LPLV to DBL ✓
    - Analysis, reporting, and publication
      - DBL to tables / graphs ✓
      - Tables / graphs to fCSR ✓

•Data Quality significantly improved through integrated data linkage and earlier & better visibility into exposure-response and patient-patient visibility

•Advanced modeling and simulation capabilities reduced need to run certain trials (e.g., add'l PK/PD studies)

•Optimized trial design and dose selection reduces possibility of re-running trials

•Use in remote locations (e.g., off-shore) significantly impacts site performance

•Real-time, Adaptive Decision Support improves POS

•15uL blood sample enables far more frequent sampling than historically possible, yielding higher integrity data, faster

•Reduces need for central lab sample shipping / analysis

•Reduces need for clinic visits

•Decentralized and automated nature of sample collection reduces number of sites / investigators Standardized real-time linkage

•Eliminates CRO and in-clinic overhead (and costs)

•Real-time data linkage reduces overhead associated with data handling / storage

•Reduced number of queries

•TPS real-time linkage impacts DBL to analysis by reducing need to consolidate various datasets

Note FPFV: First patient first visit; LPLV: Last patient last visit; DBL: Database lock
SOURCE: CMR; interviews; McKinsey analysis

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CASE STUDY B

Client ROI from POS Analyses & Recommendations

- Overview
  - Client with PoC study design question
  - Compound being used in anemia
- The Theranos Solution utilization
  - Theranos builds systems model to simulate PoC studies
  - Theranos recommends new PoC study design
- The Theranos Solution impact
  - Theranos increased probability of success from ~15% to ~80%
  - Theranos study design eNPV impact of ~$202 million

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CASE STUDY B

The Theranos Solution – Overview

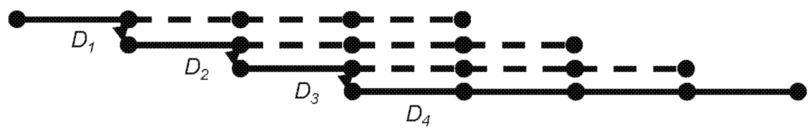
Build predictive model and use it to design proof-of-concept study.

Overview

- Theranos asked to build a predictive model for a drug with highly complex interacting physiologies and tightly limiting safety concern
- Theranos used the model to help design a proof-of-concept study that improved odds of success

Client design

- Client had originally designed a proof of concept study that included
  - Staggered dosing regimen



  - Titration regimen that had high degree of variability in patient responses (bouncing between too strong or too weak a response)

- Client had indicated that if the compound failed in the PoC study, there were 3 likely outcomes
  - Terminating compound development
  - Re-doing PoC study
  - Taking forward multiple doses forward for Phase 2b

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CASE STUDY B

The Theranos Solution – Utilization

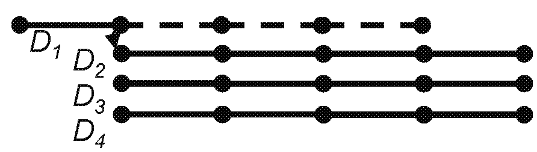
Built complex model and proposed optimized study design within 6 months.

Timeline of events

- Feb, Theranos receives request
- Mar, Theranos receives data to begin modeling
- Jun, Complex systems model built from scratch, with initial physiologically meaningful results
- July, Systems model and simulations completed with solution delivered to Client

Theranos Solution

- The Theranos Solution improved odds of success in a number of ways, including:
  - Building a complex systems model
  - Proposing a new proof of concept study design based on extensive simulation of underlying physiology including
  - Proposing a semi-parallel dosing regimen



  - Proposing a new titration regimen that reduced the likelihood of excursions above the maximum desired response and reduced the number of low-responders

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CASE STUDY B

The Theranos Solution – Impact on Success

Optimized study design increased probability of success from ~15% to ~80%.

Theranos Impact

- Probability of success through study design
  - New study design optimized dosing and titration regimens to patient responses, resulting in improved odds of success from ~15% to ~80% by causing:
    - Fewer excursions above highest dose range
    - Faster average onset of action
- Guidance to regulatory agency
  - Theranos accompanied client at meetings with regulatory agency to present new study design and rationale (and then designs for all following studies)
- Client reaction
  - Client believes The Theranos Solution study design significantly reduced likelihood of (re-)running additional studies; Estimates an impact of 18+ months saved in clinical development timeline

- ***Theranos improved Quality** by improving probability of success through optimized study design with eNPV impact of $202 million (see next slide)*
- ***Theranos also improved Speed/Cost** by reducing the need to re-do PoC study (typical PoC 18-24 months, $10-$20 million)*

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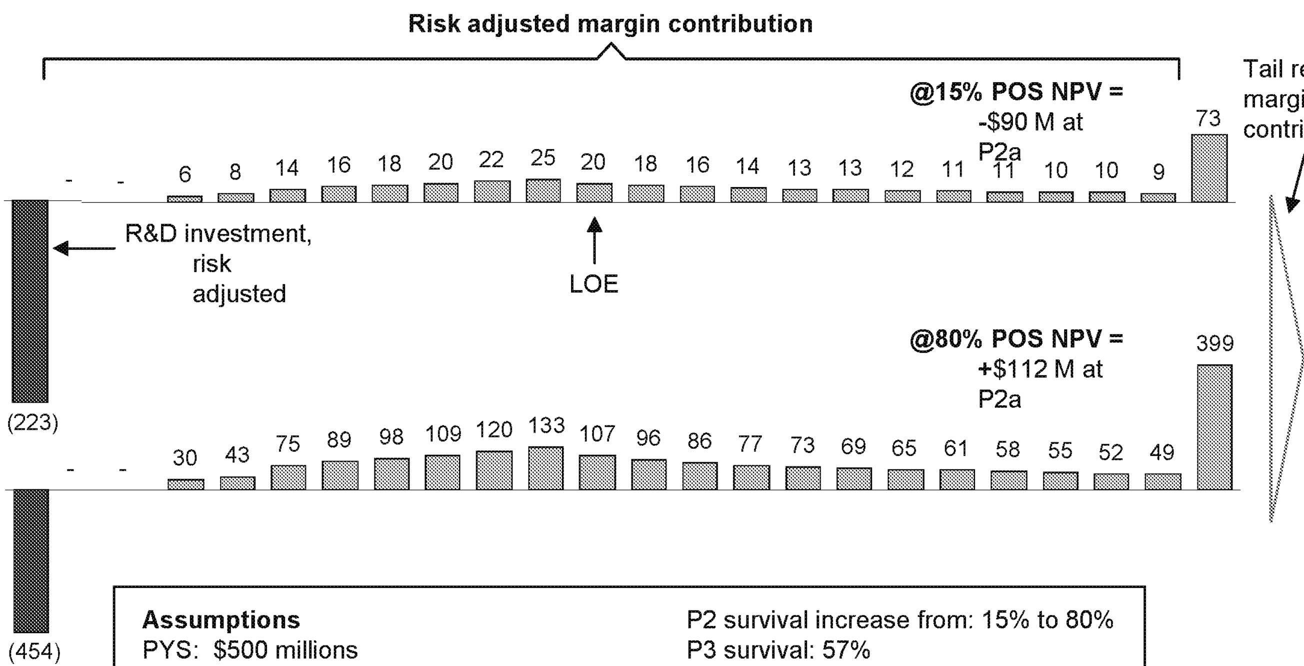
CASE STUDY B

Improving probability of survival in PoC from 15% to 80% resulted in eNPV of ~$202 million for late market drug entrant



- Discounting cash flows to beginning of P2 results in expected **NPV difference of ~$202 million** by increasing probability of success
- Return on R&D investment for successful launched compound with the profile indicated would have **increased from -3.6% to +13% (an increase in return of ~10 percentage points)**

Assumptions
PYS: $500 millions
WACC: 8%
Contribution margin: 0%, 50%, 70%, 60%
R&D capitalization rate: 9.5%
Revenue profile: Biologic

P2 survival increase from: 15% to 80%
P3 survival: 57%
Reg survival: 84%
P2 cycle time: 3.1 yrs
P3 cycle time: 2.8 yrs
Reg cycle time: 1 yr

SOURCE: PharmaProjects; DiMasi et al. 2002 Journal of Health Economics

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CASE STUDY B

The Theranos Solution – Impact on ROI

Assumptions:

- Late-to-market drug
- Potential safety issues
- Competing against established drugs
- Minimal peak year sales and success probabilities

Initial Probability of Success of 15%

- At Phase 2, value of the drug is -$90 million
- Economically unfeasible at proposed success rate
- Development is likely to be stopped
- Considering development investment to date, IRR = 3.6%

Theranos Improvement to Probability of Success of 80%

- At Phase 2, value of the drug became +$112 million
- Theranos added ~$202 million value
- Theranos effectively increases ROI to 13%.

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CASE STUDY B

Eliminating the need to repeat a single study accelerated development (estimated 18-24 months)

TPS impact

- Return on R&D investment for successful launched compound **increased ~10 percentage points**
  - Further reduction of fully loaded cost of R&D and increase of revenues from time savings
- By realizing the improvement in attrition rate across the entire portfolio versus just one compound, biopharmaceutical companies are realizing a further reduction in the fully loaded cost of R&D, because in an aggregate portfolio fewer wasted trials yield lower spend for the overall portfolio irrespective of development timelines.

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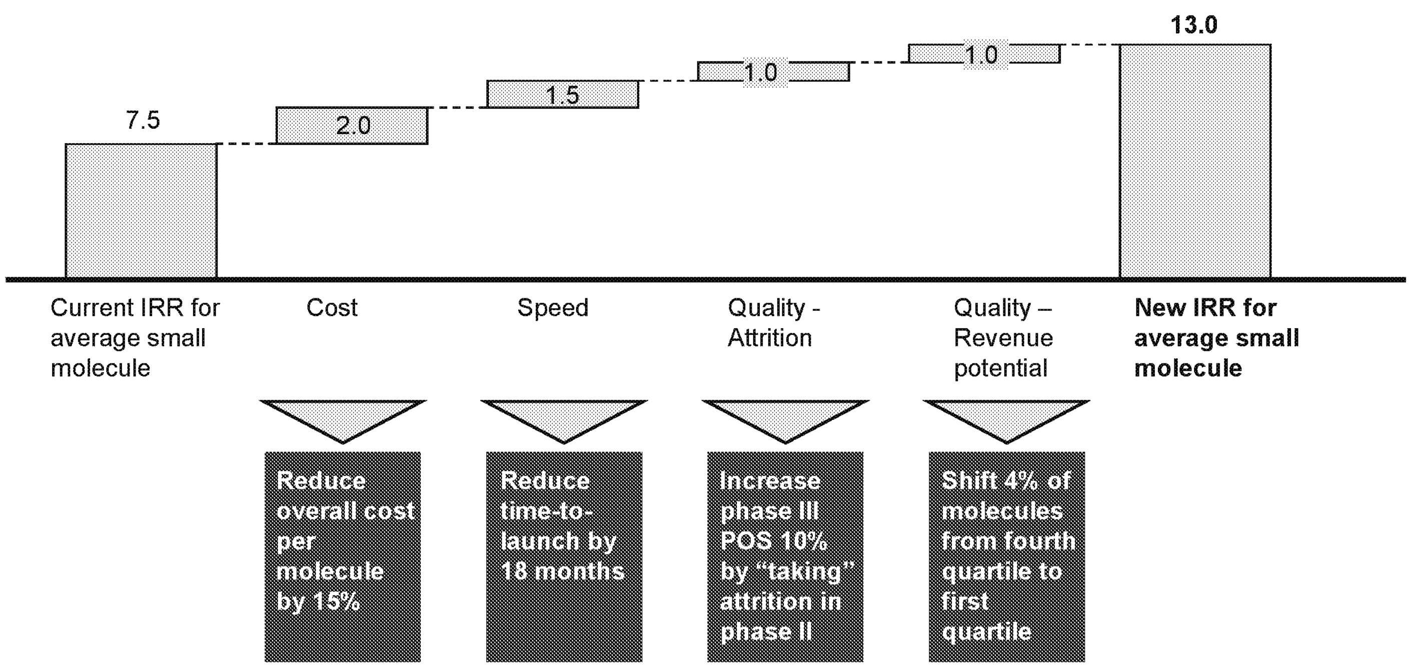
Increasing Return on R&D Investment

External research shows that pulling several operational levers can increase return on R&D investment.

Return on R&D investment
Percent



SOURCE: E. David, et al. "Pharmaceutical R&D: The Road to positive R&D returns", ***Nature Reviews Drug Discovery***

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Theranos can help achieve these improvements.

Reduce cost per molecule
- Decrease number of studies
- Decrease operating cost per patient

Reduce time to launch
- Decrease analytical time with real-time data linkage
- Reduce sample shipping from point of care (e.g., across international borders)

Increase probability of success
- Optimize decision-making around study design
- Improve dose selection due to dynamic sampling

Improve revenue per molecule
- Enable enhanced risk management
- Increase prescriber comfort for medications requiring patient monitoring

Reduce overall cost per molecule by 15%

Reduce time-to-launch by 18 months

Increase phase III POS 10% by "taking" attrition in phase II

Shift 4% of molecules from fourth quartile to first quartile

SOURCE: E. David, et al. "Pharmaceutical R&D: The Road to positive R&D returns", ***Nature Reviews Drug Discovery***

EXHIBIT 6

510(k) Premarket Notification

Device Classification Name	enzyme linked immunosorbent assay, herpes simplex virus, hsv-1
510(k) Number	K143236
Device Name	Theranos Herpes Simplex Virus-1 IgG Assay
Applicant	THERANOS, INC. 1701 PAGE MILL ROAD PALO ALTO, CA 94304
Applicant Contact	Brad Arington
Correspondent	THERANOS, INC. 1701 PAGE MILL ROAD PALO ALTO, CA 94304
Correspondent Contact	Brad Arington
Regulation Number	866.3305
Classification Product Code	MXJ
Date Received	11/12/2014
Decision Date	07/02/2015
Decision	Substantially Equivalent (SESE)
Regulation Medical Specialty	Microbiology
510k Review Panel	Microbiology
Summary	Summary
FDA Review	Decision Summary
Type	Traditional
Reviewed by Third Party	No
Combination Product	No

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Food and Drug Administration
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July 7, 2015

THERANOS, INC.
BRAD ARINGTON
ASSOCIATE DIRECTOR, REGULATORY
1701 PAGE MILL ROAD
PALO ALTO, CA 94304

Re: K143236
Trade/Device Name: Theranos Herpes Simplex Virus-1 IgG Assay
Regulation Number: 21 CFR 866.3305
Regulation Name: Herpes simplex virus serological assays
Regulatory Class: II
Product Code: MXJ
Dated: June 29, 2015
Received: June 30, 2015

Dear Mr. Arington:

This letter corrects our substantially equivalent letter of July 2, 2015.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Sally A. Hojvat -S

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K143236

Device Name
Theranos Herpes Simplex Virus-1 IgG Assay

Indications for Use *(Describe)*
The Theranos™ HSV-1 IgG Assay is a chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to herpes simplex virus type 1 (HSV-l) in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-l infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1.

The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients.

The Theranos HSV-1 IgG Assay is for use with the Theranos System which performs automated sample processing steps and result analysis.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D)   ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

510(k) SUMMARY K143236

I. GENERAL INFORMATION

Submitter:
Theranos, Inc.
1701 Page Mill Road
Palo Alto, CA 94304
Phone: 650-838-9292
Fax: 650-838-9165

Contact Person:
Brad Arington
Associate Director, Regulatory
Phone: 650-856-7304
Fax: 650-838-9165
Email: barington@theranos.com

Date Prepared: June 29, 2015

II. DEVICE INFORMATION

Trade Name: Theranos™ Herpes Simplex Virus-1 (HSV-1) IgG Assay

Common Name: HSV-1 IgG assay

Regulation Number: 21 CFR§866.3305

Regulation Name: Herpes simplex virus serological assays

Regulatory Class: Class II

Product Code: MXJ (Enzyme Linked Immunosorbent Assay, Herpes Simplex Virus, HSV-1)

Panel: Microbiology (83)

III. PREDICATE DEVICE

HerpeSelect® 1 and 2 Immunoblot IgG (K000238; Focus Diagnostics, Inc.)

IV. DEVICE DESCRIPTION

The Theranos HSV-1 IgG Assay is for use with the Theranos System. The Theranos System performs automated sample processing steps and analysis to produce the test results.

The Theranos HSV-1 IgG Assay is a three-step sandwich immunoassay with an HSV-1 glycoprotein G (gG) recombinant antigen coated surface, an anti-human IgG detection reagent conjugated to alkaline phosphatase (AP) and chemiluminescent substrate. During the first incubation step, the HSV-1 IgG antibodies present in the positive control and sample bind to the gG recombinant antigen on the coated surface. Following the first incubation step, unbound materials are removed with a wash cycle. Then the detection reagent-AP conjugate is added and during the second incubation step, the detection reagent-AP conjugate reacts with the HSV-1 IgG antibodies already bound to the capture surface. Following the second incubation, unbound materials are removed with a wash cycle. The chemiluminescent substrate is added to the capture-analyte-detection complex during the third incubation step to initiate the chemiluminescence reaction. Light generated by this reaction is detected and analyzed by the Theranos System using a calibration function to determine the cut-off index (COI) values for the sample and controls. The results for the Positive and Negative controls must be within specified limits for a run to be considered valid.

V. INDICATIONS FOR USE

The Theranos™ HSV-1 IgG Assay is a chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to herpes simplex virus type 1 (HSV-l) in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-l infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1.

The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients.

The Theranos HSV-1 IgG Assay is for use with the Theranos System which performs automated sample processing steps and result analysis.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Table 1: Similarities between the Theranos HSV-1 IgG Assay and the Predicate

Characteristic	Theranos™ HSV-1 IgG Assay **(K143236)**	Focus HerpeSelect® 1 and 2 Immunoblot IgG **(K000238)**
Intended use	Qualitative test to detect presence or absence of IgG antibodies to HSV-1 in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. Indicated for testing sexually active individuals and expectant mothers as an aid in presumptive diagnosis of HSV-1 infection. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 infection.	Qualitative test to detect presence or absence of IgG antibodies to HSV-1 and HSV-2 in human sera. Indicated for testing sexually active adults or expectant mothers as an aid in presumptive diagnosis of HSV-1 and HSV-2 infection. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2 infection.

Table 2: Differences between the Theranos HSV-1 IgG Assay and the Predicate

Characteristic	Theranos™ HSV-1 IgG Assay **(K143236)**	Focus HerpeSelect® 1 and 2 Immunoblot IgG **(K000238)**
Specimen Types	• Venous serum, • K2-EDTA anticoagulated human plasma from venous blood, • Human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes	• Venous Serum
Type of Assay	Chemiluminescent immunoassay	Nitrocellulose Immunoblot
Sample Handling	Automated sample handling/processing	Manual sample handling/processing
Capture Reagent	HSV-1 recombinant antigen (gG1)	HSV-1/HSV-2 antigen immobilized on nitrocellulose membrane

VII. PERFORMANCE

To characterize performance of the Theranos HSV-1 IgG immunoassay the following studies were conducted:

<u>Precision – CLIA Laboratory Model, Venous Serum</u>

A study for estimating the precision of the Theranos HSV-1 IgG Assay for venous serum samples in a CLIA Laboratory model was performed by testing a panel of 6 serum samples spanning the analytical range [negative (A), high negative (B), equivocal (C), low positive (D), moderate positive (E), and positive (F)]. The precision study was conducted at one site with thirty five (35) TSPU devices, three (3) lots of cartridges and sixteen (16) operators in total. The study duration was 13 days in total. Details of the study design for different samples are presented in Table 3 below.

Table 3: Design of Precision Study: Numbers of Replicates, Devices, Days and Operators

Panel Member	Valid Replicates				No. of Devices	No. of Days	No. of Operators	No. of Invalid Replicates
	Total	Lot 1	Lot 2	Lot 3				
A (Neg.)	91	26	38	27	35	7	14	3
B (High Neg.)	88	24	37	27	28	7	14	2
C (Equivocal)	78	27	44	8*	35	8	16	3
D (Low Pos.)	80	25	27	28	11	2	4	4
E (Mod. Pos.)	64	25	13	26	13	2	6	1
F (Pos.)	69	25	19	25	15	2	4	3

*Sufficient cartridges from reagent lot #3 were not available.

Results of the precision study are presented in Table 4.

Table 4: Summary of Precision Study Results

Panel Member	Mean (COI)		Repeatability (same device, same lot)	Between-device	Between-lot	Precision (same device, different lot)	Precision (different device, same lot)	Precision (different device, different lot)
A (Neg.)	0.425	SD	0.049	0.007	0.000	0.049	0.049	0.049
		%CV	11.5%	1.6%	0%	11.5%	11.6%	**11.6%**
B High Neg.)	0.648	SD	0.086	0.011	0.029	0.091	0.087	0.092
		%CV	13.3%	1.7%	4.5%	14.1%	13.4%	**14.2%**
C Equivocal)	1.016	SD	0.093	0.062	0.065	0.113	0.112	0.129
		%CV	9.1%	6.1%	6.4%	11.1%	11.0%	**12.7%**
D (Low Pos.)	1.727	SD	0.208	0.098	0.013	0.208	0.230	0.230
		%CV	12.0%	5.7%	0.8%	12.0%	13.3%	**13.3%**
E (Mod. Pos.)	3.809	SD	0.305	0.276	0.108	0.324	0.411	0.425
		%CV	8.0%	7.3%	2.8%	8.5%	10.8%	**11.2%**
F (Pos.)	8.996	SD	0.807	0.437	0.000	0.807	0.918	0.918
		%CV	9.0%	4.9%	0.0%	9.0%	10.2%	**10.2%**

Table 5 presents percent of invalid results and percents of negative, equivocal and positive among valid results for each sample.

Table 5: Percent of Invalid Results and Percents of Negative, Equivocal and Positive among Valid Results

Panel Member	Mean (COI)	Number of Replicates	Percent of Invalid	Percent of Negative among Valid	Percent of Equivocal among Valid	Percent of Positive among Valid
A (Neg.)	0.425	94	3.2% (3/94)	100% (91/91)		
B (High Neg.)	0.648	90	2.2% (2/90)	100% (88/88)		
C (Equivocal)	1.016	81	3.7% (3/81)	17.9% (14/78)	60.3% (47/78)	21.8% (17/78)
D (Low Pos.)	1.727	84	4.8% (4/84)			100% (80/80)
E (Mod. Pos.)	3.809	65	1.5% (1/65)			100% (64/64)
F (Pos.)	8.996	72	4.2% (3/72)			100% (69/69)

The results of the study demonstrate that the precision of the Theranos HSV-1 IgG Assay (including different TSPU devices, different lots of cartridges, and different operators) when performed in a CLIA Laboratory was in the range 10.2% to 14.2%.

Precision – CLIA Laboratory Model, Fingerstick Whole Blood

A study for estimating the precision of the Theranos HSV-1 IgG Assay for fingerstick whole blood samples in a CLIA Laboratory model was performed by testing a panel of 3 fingerstick plasma samples spanning the analytical range [high negative (P), equivocal (Q), moderate positive (R)]. The precision study was conducted at one site with thirty six (36) TSPU devices, three (3) lots of cartridges and nine (9) operators in total. The study duration was 4 days in total. Details of the study design for different samples are presented in Table 6 below.

Table 6: Design of Precision Study: Numbers of Replicates, Devices, Days and Operators

Panel Member	Valid Replicates				No. of Devices	No. of Days	No. of Operators	No. of Invalid Replicates
	Total	Lot 1	Lot 2	Lot 3				
P (High Neg.)	168	56	56	56	30	4	9	3*
Q (Equivocal)	168	56	56	56	29	4	9	2*
R (Mod. Pos.)	168	56	56	56	27	4	9	2*

*All invalid replicates were repeated

Results of the precision study are presented in Table 7.

Table 7: Summary of Precision Study Results

Panel Member	Mean (COI)		Repeatability (same device, same lot)	Between-device	Between-lot	Precision (same device, different lot)	Precision (different device, same lot)	Precision (different device, different lot)
P (High Neg.)	0.888	SD	0.083	0.006	0.050	0.096	0.083	0.097
		%CV	9.3%	0.7%	5.6%	10.9%	9.3%	**10.9%**
Q (Equivocal)	1.047	SD	0.094	0.025	0.069	0.117	0.098	0.119
		%CV	9.0%	2.4%	6.6%	11.1%	9.3%	**11.4%**
R (Mod. Pos.)	3.241	SD	0.342	0.122	0.157	0.377	0.363	0.396
		%CV	10.6%	3.8%	4.9%	11.6%	11.2%	**12.2%**

Table 8 presents percent of invalid results and percents of negative, equivocal and positive among valid results for each sample.

Table 8: Percents of Positive, Equivocal, Negative and Invalid Results

Panel Member	Mean (COI)	Number of Replicates	Percent of Invalid	Percent of Negative among Valid	Percent of Equivocal among Valid	Percent of Positive among Valid
P (High Neg.)	0.888	171	1.8% (3/171)	58.3% (98/168)	40.5% (68/168)	1.2% (2/168)
Q (Equivocal)	1.047	170	1.2% (2/170)	6.5% (11/168)	63.1% (106/168)	30.4% (51/168)
R (Mod. Pos.)	1.016	170	1.2% (2/170)			100% (168/168)

The results of the study demonstrate that precision of the Theranos HSV-1 IgG Assay (including different TSPU devices, different lots of cartridges, and different operators) when performed in a CLIA Laboratory was in the range from 10.9% to 12.2%.

Reproducibility

A study designed to process multiple fingerstick whole blood samples from individual subjects was performed to evaluate the reproducibility of the Theranos HSV-1 IgG Assay when used with Theranos Capillary Tubes and Nanotainer Tubes. The study was conducted at 3 collection sites with 10 subjects at each site. From each of 30 subjects, 9 Capillary Tubes and Nanotainer Tubes from 3 manufacturing lots (i.e. 3 Capillary Tubes and Nanotainer Tubes per lot) and 2 serum separator tubes (SSTs) were collected. Each subject had the following measurements:

- Each of the 9 Capillary Tubes and Nanotainer Tubes was tested. These data were used for evaluation of between-Capillary Tubes and Nanotainer Tubes imprecision, between-lot imprecision and total imprecision that includes between-Capillary Tubes and Nanotainer Tubes and between-lot imprecisions.
- One Nanotainer Tube (from one of the 3rd lot of Capillary Tubes and Nanotainer Tubes for each subject) was tested in duplicate via recovering a sample from one

Capillary Tubes and Nanotainer Tubes and transferring a sample to another Capillary Tubes and Nanotainer Tubes. These data were used for evaluation of within-Capillary Tubes and Nanotainer Tubes imprecision.

- Each of the 2 SSTs was tested. These data were used for evaluation of between-SST imprecision.

For samples with mean COI value at the baseline ≥0.5, percent differences were calculated and for samples with mean COI value at the baseline <0.5, differences were calculated. Table 9 summarizes the results of the reproducibility study broken down by collection site and by high or low COI subjects; the variability metrics are averaged across all subjects within the site.

Table 9: Summary of Results of the Reproducibility Study

Collection Site	Subjects	Capillary Tubes and Nanotainer Tubes				SST
		Within-Capillary Tubes and Nanotainer Tubes (%CV or SD)	Between-Capillary Tubes and Nanotainer Tubes (%CV or SD)	Between-Lot (%CV or SD)	Total (%CV or SD)	Between-SST (%CV or SD)
1	6 subjects with COI values 1.4 – 13.5	%CV=6.0%	%CV = 9.0%	%CV = 6.8%	%CV=12.6%	%CV=9.6%
	4 subjects with COI values 0.03-0.28	SD=0.008	SD = 0.015	SD = 0.016	SD = 0.024	SD=0.11
2	7 subjects with COI values 1.6 – 16.8	%CV=8.2%	%CV=9.2%	%CV=3.2%	%CV=10.8%	%CV=12.5%
	3 subjects with COI values 0.07-0.19	SD=0.009	SD=0.011	SD=0.008	SD=0.015	SD=0.019
3	5 subjects with COI values 4.5 – 14.3	%CV=8.2%	%CV=8.1%	%CV=6.0%	%CV=11%	%CV=12.4%
	5 subjects with COI values 0.02-0.32	SD=0.08	SD=0.019	SD=0.013	SD=0.025	SD=0.021
Combined	18 subjects with COI values 1.4-16.8	%CV=7.5%	%CV=8.8%	%CV=5.2%	%CV=11.4%	%CV=11.5%
	12 subjects with COI values 0.02-0.32	SD=0.008	SD=0.015	SD=0.013	SD=0.022	SD=0.017

- Within-Capillary Tubes and Nanotainer Tubes imprecision was %CV=7.5% for aggregated subjects with mean COI ≥ 0.5 and SD=0.008 for aggregated subjects with a mean COI < 0.5.

- Total imprecision including between-Capillary Tubes and Nanotainer Tubes and between-lot imprecisions was %CV= 11.4% for aggregated subjects with a mean COI ≥ 0.5 and SD=0.022 for aggregated subjects with a mean COI < 0.5.

- Between-serum separator tubes imprecision was %CV=11.5% for aggregated subjects with a mean COI ≥ 0.5 and SD=0.017 for aggregated subjects with a mean COI < 0.5.

Analyte Stability

An analyte stability study was performed to characterize the stability of HSV-1 IgG in clinical matrices as measured by the Theranos HSV-1 IgG Assay under different sample storage conditions and time periods. A summary of analyte storage conditions and durations for different sample types and matrices claimed for the Theranos HSV-1 IgG Assay is presented in Table 10.

Table 10: Summary of Analyte Storage Conditions and Durations

Condition	**Venous Serum**	**Venous K2-EDTA Plasma**	**Fingerstick K2-EDTA Plasma**	**Fingerstick K2-EDTA Whole Blood**
Stored at 2-8°C	48 hr	48 hr	48 hr	48 hr
Stored at room temperature (20-25°C)	6 hr	6 hr	6 hr	6 hr
Stored at -20°C	1 week	1 week	1 week	N/A
Freeze/thaw cycles	3	3	3	N/A

Within 2 hours after collection, one aliquot of each sample type or matrix was tested with the Theranos HSV-1 IgG Assay in duplicate, to establish the value at baseline. The samples were stored in Nanotainer Tubes under the appropriate conditions. Comparison of an average of two replicates at the predetermined time points with the average of two replicates at baseline was performed. For samples with a mean COI value at the baseline ≥0.5, percent differences were calculated and for samples with a mean COI value at the baseline <0.5, differences were calculated.

Acceptance criteria were as follows: i) a difference averaged over all samples with baseline mean COI value ≥0.5 must be less than ±10% and a difference averaged over all samples with baseline COI mean <0.5 must be less than 0.02 and ii) for each sample, an observed difference must be less than 15% for the samples with baseline mean COI value ≥0.5 and must be less than 0.08 for the samples with baseline mean COI value <0.5 (the range of differences expected if there is no effect of storage on the HSV-1 IgG analyte). The results are summarized in Table 11.

Table 11: Summary of Mean Absolute Difference Measures for all Storage Conditions and Sample Types or Matrices

	Sample Type and Matrix	Samples with a Baseline COI < 0.5		Samples with a Baseline COI > 0.5	
		Difference averaged over all samples	The largest observed difference among samples	Percent difference averaged over all samples	The largest observed percent difference among samples
Stored at 2-8C, 48 hrs	Venous serum	0.006	0.006	1.0%	13.6%
	Venous K2-EDTA plasma	-0.007	-0.007	2.3%	13.3%
	Fingerstick K2-EDTA plasma from whole blood	0.003	0.003	-0.4%	13.9%
Stored at -20, 1 week	Venous serum	0.008	0.015	0.8%	13.3%
	Venous K2-EDTA plasma	0.003	0.005	-1.0%	12.7%
	Fingerstick K2-EDTA plasma from whole blood	0.001	0.008	1.8%	-13.9%
Freeze thaw cycles, n=3	Venous serum	0.007	0.021	-0.1%	13.6%
	Venous K2-EDTA plasma	0.021	0.037	-1.7%	-13.4%
	Fingerstick K2-EDTA plasma from whole blood	0.006	0.022	-1.0%7	13.6%
Stored at room temp, 6 hrs	Venous serum	-0.001	-0.011	-3.2%	-11.9%
	Venous K2-EDTA plasma	0.002	0.022	0.1%	13.7%
	Fingerstick K2-EDTA plasma from whole blood	-0.004	-0.026	1.1%	13.9%

Interfering Substances

A study was designed and performed (in accordance with CLSI EP07-A2) to evaluate the performance of the Theranos HSV-1 IgG Assay in the presence of potentially interfering substances to assess the impact of these endogenous substances and commonly used drugs on the performance of the Theranos HSV-1 IgG Assay. Interferents were tested with three serum samples (negative (mean COI 0.024), high negative (mean COI 0.77) and low positive (mean COI 1.52)) that were contrived by using a high positive sample and diluting it with pooled negative serum. Samples were spiked with the interferent at levels shown in Table 12. Each serum pool was tested in duplicate.

For the low positive and the high negative pools, the acceptance criteria were a mean recovery within +/- 20% of the value of the unspiked sample (i.e., in the absence of the potential interferent or drug). All low positive and high negative samples showed a signal change of less than 15% for all interfering substances. All positive samples remained positive and all negative samples remained negative upon spiking of drug or other interferents. For the negative pool, the acceptance criterion was a deviation of less than 0.02 COI. All negative samples showed a mean deviation of ≤0.02 COI, except Intralipid. Intralipid spikes did not show any effect on recovery for near cut-off samples, high negative and low positive samples.

Table 12: Summary of Interfering Substances Studies: Endogenous Interferents and Drug Interferents

		Negative Pool		High Negative Pool		Low Positive Pool	
Interferent	**Level**	**Mean COI**	**ΔCOI**	**Mean COI**	**% Recovery**	**Mean COI**	**% Recovery**
Hemoglobin	1000 mg/dL	0.025	0.00	0.69	90	1.71	113
Bilirubin	20 mg/dL	0.024	0.00	0.68	88	1.61	106
Intralipid	2000 mg/dL	0.053	0.03	0.81	105	1.60	105
Acetylcysteine	150 mg/L	0.019	-0.004	0.68	88	1.40	92
Ampicillin-Na	1000 mg/L	0.025	0.001	0.76	99	1.44	95
Ascorbic acid	300 mg/L	0.027	0.003	0.75	97	1.67	110
Ca-Dobesilate	200 mg/L	0.027	0.004	0.70	91	1.51	99
Cyclosporine	5 mg/L	0.031	0.008	0.74	97	1.53	101
Cefoxitin	2500 mg/L	0.027	0.003	0.74	97	1.52	100
Heparin	5000U	0.020	-0.003	0.80	103	1.52	100

		Negative Pool		High Negative Pool		Low Positive Pool	
Interferent	**Level**	**Mean COI**	**ΔCOI**	**Mean COI**	**% Recovery**	**Mean COI**	**% Recovery**
Levodopa	20 mg/L	0.030	0.006	0.68	88	1.42	94
Methyldopa+1.5h20	20 mg/L	0.024	0.000	0.74	97	1.37	90
Metronidazole	200 mg/L	0.039	0.016	0.74	96	1.38	91
Phenylbutazone	400 mg/L	0.021	-0.002	0.74	96	1.42	94
Doxycycline	50 mg/L	0.024	0.000	0.71	92	1.35	89
Acetylsalicylic acid	1000 mg/L	0.026	0.002	0.75	97	1.37	90
Rifampicin	60 mg/L	0.014	-0.009	0.69	90	1.35	89
Acetaminophen	200 mg/L	0.034	0.010	0.64	83	1.68	111
Control		0.024	0.000	0.77	100	1.52	100

Cross-reactivity

A study was performed to evaluate the performance of the Theranos HSV-1 IgG Assay in the presence of IgG antibodies against twenty-one (21) infectious agents defined as potential cross-reactants in the FDA guidance on HSV serological assays. Banked serum samples confirmed positive for IgG against the infectious agents of interest were acquired from commercial vendors. At least three (3) samples, independently confirmed as positive for that agent and negative for HSV-1 IgG on the reference method, were tested on the Theranos HSV-1 IgG Assay in order to rule out cross-reactivity of the Theranos HSV-1 IgG Assay with IgG against a potential cross reactant. The results of this study are displayed in Table 13.

Table 13: Summary of Cross-reactivity Study on Theranos HSV-1 IgG Assay

Organism/Condition	No.	Reference HSV-1 Assay	Theranos HSV-1 Positive	Theranos HSV-1 Negative	Theranos HSV-1 Equivocal
Epstein Barr Virus (IgG)	6	Negative	0	6	0
Epstein Barr Virus (IgM)	1	Negative	0	1	0
HPV	4	Negative	0	4	0
Rubella (IgG)	13	Negative	0	13	0
HSV-2 (IgG)	40	Negative	0	40	0
HAMA samples	4	Negative	0	4	0
Treponema pallidum	8	Negative	0	7	1*
Rheumatoid Factor (RF)	8	Negative	1**	7	0
Anti-nuclear antibody (ANA)	8	Negative	0	8	0
Sjogren's Syndrome	3	Negative	0	3	0
CMV (IgG)	5	Negative	0	5	0
CMV (IgM)	2	Negative	0	2	0
Chlamydia trachomatis (IgG)	10	Negative	0	10	0
HCV (IgG)	3	Negative	0	3	0
HBsAg	3	Negative	0	3	0
VZV IgG	5	Negative	0	5	0
Measles IgG	5	Negative	0	5	0
HIV-1 (IgG)	4	Negative	0	4	0
Toxoplasma IgG	4	Negative	0	4	0
Candida albicans Ag	3	Negative	0	3	0
Systemic Lupus	3	Negative	0	3	0

*Systematic cross-reactivity ruled out (7/8 samples in same category tested negative)
**Confirmed as positive upon retest by Theranos HSV-1 assay; systematic cross-reactivity ruled out (7/8 samples in same category tested negative)

Assay Cut-off

A study was performed to establish the cut-off and the limits of the equivocal zone for the Theranos HSV-1 IgG Assay using 192 serum samples. Then 120 independent serum samples were analyzed to validate the established cut-off. The calibrators were assigned COI values based on the established assay cut-off, the cut-off for positive results a COI of 1.1 and cut-off for negative results a COI of 0.9. The results of the cut-off validation study are displayed in Table 14 below.

Table 14: Performance of Selected Cut-off on Independent Sample Set

Agreement Classification	Percent Agreement	95% Confidence Interval
NPA	96.0% (47/49)	86.3-98.9
PPA	97.1% (69/71)	90.3-99.2

Fingerstick Plasma – CLIA Laboratory Model

To demonstrate the performance of the Theranos HSV-1 IgG Assay for fingerstick whole blood samples collected at 3 Theranos Patient Service Centers (TPSCs) and processed at the CLIA-certified laboratory.

At each site, fingerstick whole blood samples were collected into a pair of Theranos Capillary Tubes and Nanotainer Tubes, and venous samples were collected into serum tubes from each of 20, 16 and 25 adult subjects at three collection sites.

Samples were shipped refrigerated to the Theranos CLIA-certified laboratory in Palo Alto, CA. Upon receipt, fingerstick whole blood samples in the Nanotainer Tubes were centrifuged at 1200g for 5 minutes. Plasma was extracted and processed and analyzed on the Theranos System. All samples were processed or frozen as plasma within 48 hours of draw.

A summary of the performance information is shown in Table 15.

Table 15: Summary of Method Comparison for Samples Collected at 3 Theranos Patient Service Centers

		Reference Result	
		POS	NEG
Theranos Result	POS	38	0
	NEG	1	22

	Point Estimate	95% Confidence Interval
Sensitivity	97.4% (38/39)	86.8 – 99.6
Specificity	100% (22/22)	85.1 – 100

Matrix Comparison

The effect of anticoagulants and different sample types (fingerstick and venous) on the performance of the Theranos HSV-1 IgG Assay was determined by comparing matched venous serum, venous K2-EDTA plasma, and fingerstick K2-EDTA plasma samples from 70 donors. Forty-three matched sample sets were contrived to have analyte values close to the cut-off. The acceptance criterion was a recovery of positive plasma samples within ± 20% of the corresponding serum reference value (serum drawn into primary tubes without gel). For negative samples, the acceptance criteria was a difference of <= 0.02 COI from the corresponding serum value. All anticoagulant–treated plasma samples met this criterion. Weighted Deming regression was performed. The slope and an intercept of the regression line and their 95% confidence intervals along with correlation coefficients are shown in Table 16 and a graphical depiction is shown in Figure 1.

Table 16: Summary of Weighted Deming Regression Analysis Performed on Matrix Equivalency Data for Venous Plasma and Fingerstick Plasma Samples

Sample Type/Matrix	Correlation coefficient	Slope	95% confidence interval on slope	Intercept	95% confidence interval on intercept
Venous plasma	0.992	0.993	[0.967, 1.019]	0.000	[-0.003, 0.003]
Fingerstick plasma	0.995	1.009	[0.973, 1.044]	-0.003	[-0.006, -0.001]

Figure 1: Regression Analysis for Matrix Comparison Study

<u>Expected Values</u>

The Theranos HSV-1 IgG Assay was used to evaluate the prevalence of HSV-1 IgG antibodies in individuals for whom an HSV-1 IgG test was ordered by a physician including pregnant women. The study population for the Theranos HSV-1 IgG Assay consisted of a total of 558 subjects, with 260 sexually active adults and 298 individuals identified as pregnant women. The result for 1 out of the 558 subjects is not reported, as indicated in Table 17 (1 subject), giving a total of 557 subjects. The data for the intended use population (557 specimens) have been summarized according to age group in decades, gender, number of reactive results, number of equivocal results, and number of non-reactive results. The data for the intended use population have been summarized in Table 17 (259 specimens from sexually active adult subjects) and Table 18 (298 specimens from pregnant subjects).

Table 17: Expected Results for Theranos HSV-1 IgG Assay in Sexually Active Adult Subjects

Age Range	Gender	Reactive	Equivocal	Non-Reactive
		N/Total (%)	N/Total (%)	N/Total (%)
16 to 19	Male	0/0 (0)	0/0 (0)	0/0 (0)
16 to 19	Female	1/4 (25)	0/4 (0)	3/4 (75)
20 to 29	Male	8/18 (44.4)	1/18 (5.6)	9/18 (50)
20 to 29	Female	29/73 (39.7)	0/73 (0)	44/73 (60.3)
30 to 39	Male	5/10 (50)	0/10 (0)	5/10 (50)
30 to 39	Female	33/62 (53.2)	0/62 (0)	29/62 (46.8)
40 to 49	Male	5/10 (50)	0/10 (0)	5/10 (50)
40 to 49	Female	16/27 (59.3)	0/27 (0)	11/27 (40.7)
50 to 59	Male	17/20 (85)	0/20 (0)	3/20 (15)
50 to 59	Female	9/11 (81.8)	0/11 (0)	2/11 (18.2)
60 to 69	Male	5/6 (83.3)	0/6 (0)	1/6 (16.7)
60 to 69	Female	5/10 (50)	1/10 (10)	4/10 (40)
70 to 79	Male	3/4 (75)	0/4 (0)	1/4 (25)
70 to 79	Female	1/3 (33.3)	0/3 (0)	2/3 (66.7)
80 to 89	Male	0/0 (0)	0/0 (0)	0/0 (0)
80 to 89	Female	1/1 (100)	0/1 (0)	0/1 (0)
Total*		138/259 (53.3)	2/259 (0.8)	119/259 (45.9)

*1 sample not reported since age information was not available

Table 18: Expected Results for Theranos HSV-1 IgG Assay in Pregnant Subjects

Age Range	Gender	Reactive	Equivocal	Non-Reactive
		N/Total (%)	N/Total (%)	N/Total (%)
18 to 19	Female	13/13 (100)	0/13 (0)	0/13 (0)
20 to 29	Female	114/175 (65.1)	1/175 (0.6)	60/175 (34.3)
30 to 39	Female	61/104 (58.7)	0/104 (0)	43/104 (41.3)
40 to 49	Female	5/6 (83.3)	0/6 (0)	1/6 (16.7)
Total		193/298 (65)	1/298 (0.3)	104/298 (35)

The hypothetical positive and negative predictive values (PPV, NPV) for the two intended use populations are shown in Table 19. The calculations are based on the specificity and sensitivity values for the Theranos HSV-1 IgG Assay determined in the clinical study;

1. Specificity of 97.4% and Sensitivity of 95.1% in sexually active adults
2. Specificity of 95.2% and Sensitivity of 97.4% in pregnant women

Table 19: Hypothetical Predictive Values

Prevalence (%)	Sexually Active Adults		Pregnant Women	
	PPV (%)	NPV (%)	PPV (%)	NPV (%)
50	93.8	92.6	92.1	91.7
45	93.2	93.2	91.3	92.4
40	92.4	93.8	90.3	93.0
35	91.4	94.2	89.1	93.5
30	90.1	94.6	87.5	94.0
25	88.3	94.9	85.3	94.3
20	85.8	95.2	82.3	94.7
15	82.0	95.5	77.7	95.0
10	75.2	95.7	69.9	95.2
5	60.2	96.0	53.8	95.5

Clinical Performance in the Intended Use Populations (CLIA Laboratory Model)

A clinical study was conducted to characterize the performance of the Theranos HSV-1 IgG Assay in the Theranos CLIA-certified Laboratory in comparison to the FOCUS HerpeSelect Immunoblot (as the reference method for performance analysis).

Prospectively collected, archived venous serum samples collected from pregnant women and sexually active adults (18 years and older) who had a prescription for a HSV-1 IgG test. Samples were obtained from multiple specimen sources covering 10 US states and Mexico.

The equivocal results on the Focus HerpeSelect Immunoblot (that repeatedly tested equivocal) were resolved using a validated western blot reference test (University of Washington, Seattle) as per the instructions of the package insert for the reference method. Ten samples from the sexually active adult sub-population tested initially equivocal on the Focus HerpeSelect Immunoblot and were resolved by the University of Washington western blot as 2 negatives and 7 positives. One sample was not resolved. There were no samples in the sexually active adult sub-population that returned an invalid result.

In the pregnant women sub-population, 8 samples tested initially equivocal on the Focus HerpeSelect immunoblot. Of these, 4 samples could not be resolved by the University of Washington western blot due to insufficient volume availability. Of the remaining 4, 1 (one) were resolved as negative and 3 as positive. There were 3 samples that returned an invalid result on the Theranos HSV-1 IgG test. These samples were rerun and resulted in

valid results included in the analysis. The clinical performance results are summarized in Tables 20 and 21.

Table 20: Summary of Theranos HSV-1 IgG Assay Performance in Sexually Active Adult Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	137	0	2	139
	Equivocal	1	0	1	2
	Negative	5	1	113	119
	Total	143	1	116	***260***

	Point Estimate	95% Confidence Interval
Sensitivity	95.1% (137/144)	90.3-97.6
Specificity	97.4% (113/116)	92.7-99.1

Table 21: Summary of Theranos HSV-1 IgG Assay Performance in Pregnant Women Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	188	1	4	193
	Equivocal	0	1	0	1
	Negative	2	2	100	104
	Total	190	4	104	***298***

	Point Estimate	95% Confidence Interval
Sensitivity	97.9% (188/192)	94.8-99.2
Specificity	95.2% (100/105)	89.3-98.0

CDC Panel Testing

The objective of this study was to demonstrate agreement of the Theranos HSV-1 IgG Assay with the CDC panel. A panel of well characterized serum samples (n=100) was obtained from the U.S. Centers for Disease Control and Prevention (CDC). The CDC sample panel was tested with the HSV-1 IgG Assay and the results obtained by Theranos were sent to the CDC for confirmation. The panel consisted of 54 positives and 46 negatives. The Theranos HSV-1 IgG Assay demonstrated 100% agreement with the results provided by the CDC.

Low Prevalence Population

Serum samples were collected from a low prevalence population: Individuals who are not sexually active, and without a recent or current sexually transmitted disease (Hepatitis, Syphilis, HIV, HPV, Trichomonas, Chlamydia, Gonorrhoeae) as determined in an interview. Performance of the assay on this population is summarized in Table 22. The Samples were obtained from multiple specimen sources covering 10 US states and Mexico.

Table 22: Summary of Theranos HSV-1 IgG Assay Performance with Low Prevalence Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	32	0	0	32
	Equivocal	0	0	0	0
	Negative	0	1	49	50
	Total	32	1	49	***82***

	Point Estimate	95% Confidence Interval
Sensitivity	97.0% (32/33)	84.7-99.5
Specificity	100% (49/49)	92.7-100

CONCLUSIONS

The results of the analytical and clinical performance studies submitted in this premarket notification are complete and demonstrate that the Theranos HSV-1 IgG Assay meets the established specifications necessary for consistent performance during intended clinical use. The results support a decision that the Theranos Herpes Simplex Virus-1 (HSV-1) IgG Assay is substantially equivalent to the predicate.

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K143236

B. Purpose for Submission:

New device

C. Measurand:

HSV-1 specific IgG antibodies

D. Type of Test:

Chemiluminescent immunoassay

E. Applicant:

Theranos, Inc.

F. Proprietary and Established Names:

Theranos™ Herpes Simplex Virus-1 (HSV-1) IgG Assay

G. Regulatory Information:

1. Regulation section:

   866.3305

2. Classification:

   Class II Special Controls

3. Product code:

   MXJ

4. Panel:

   Microbiology

H. Intended Use:

1. Intended use(s):

   The Theranos™ HSV-1 IgG Assay is a chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to herpes simplex virus type 1 (HSV-l) in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-l infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1.

   The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients.

   The Theranos HSV-1 IgG Assay is for use with the Theranos System which performs automated sample processing steps and result analysis.

2. Indication(s) for use:

   Same as the intended use

3. Special conditions for use statement(s):

   The Theranos HSV-1 IgG Assay is for prescription use only in accordance with 21 CFR 801.109

4. Special instrument requirements:

   Theranos Sample Processing Unit (TSPU)

I. Device Description:

The Theranos anti-HSV-1 IgG Assay is a three-step sandwich chemiluminescent immunoassay with an HSV-1 glycoprotein G (gG) recombinant antigen coated surface, an anti-human IgG detection reagent conjugated to alkaline phosphatase (AP) and chemiluminescent substrate. The Theranos HSV-1 IgG Assay reagents are packaged in a ready-to-use pouched cartridge and are designed to be used directly. No additional preparation of reagents is required. The Theranos HSV-1 IgG Assay is intended to be run on the Theranos™ TSPU Device only. The Theranos TSPU Device performs automated sample processing and signal detection. Raw data collected are analyzed by the Theranos System under the oversight of the Theranos CLIA-certified laboratory. The Theranos TSPU is intended to be used only with Theranos assay cartridges.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Focus HerpeSelect® 1 and 2 Immunoblot IgG

2. Predicate 510(k) number(s):
K000238

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for use	The Theranos™ HSV-1 IgG Assay is a chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to herpes simplex virus type 1 (HSV-l) in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-l infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1. The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients. The Theranos HSV-1 IgG Assay is for use with the Theranos System which	Focus Diagnostics' HerpeSelect® 1 and 2 Immunoblot IgG test is intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-1 and HSV-2 in human sera. The test is indicated for testing sexually active adults or pregnant women for aiding in the presumptive diagnosis of HSV-1 and HSV-2 infection. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2 infection. The performance of this assay has not been established for use in a pediatric population, for neonatal screening, for testing of immuno-compromised patients, for use by a point of care facility or for use with automated equipment.

Similarities		
Item	Device	Predicate
	performs automated sample processing steps and result analysis.	

Differences		
Item	Device	Predicate
Specimen Type	Venous serum, K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes	Venous Serum
Technology	Chemiluminescent immunoassay	Nitrocellulose Immunoblot

K. Standard/Guidance Document Referenced (if applicable):

Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Herpes Simplex Virus Types 1 and 2 Serological Assays (issued August 9, 2011)

Guidance for Industry and FDA Staff: Assay Migration Studies for In Vitro Diagnostic Devices (issued April 25, 2013)

Guidance for Industry and FDA Staff: Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests (issued March 13, 2007)

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

CLSI EP07-A2, Interference Testing in Clinical Chemistry;. Approved Guideline—Second Edition

CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline—Second Edition

CLSI EP14-A2, Evaluation of Matrix Effects; Approved Guideline—Second Edition

CLSI EP17-A, Evaluation of Detection Capability for Clinical. Laboratory Measurement Procedures; Approved Guideline—Second Edition

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic. Reagents; Approved Guideline

CLSI GP34-A, Validation and Verification of Tubes for. Venous and Capillary Blood Specimen. Collection; Approved Guideline

Test Principle:

During the first incubation step, the HSV-1 IgG antibodies present in the positive control and sample bind to the gG recombinant antigen on the coated surface. Following the first incubation step, unbound materials are removed with a wash cycle. Then the detection reagent-AP conjugate is added and during the second incubation step, the detection reagent-AP conjugate reacts with the HSV-1 IgG antibodies already bound to the capture surface. Following the second incubation, unbound materials are removed with a wash cycle. The chemiluminescent substrate is added to the capture-analyte-detection complex during the third incubation step to initiate the chemiluminescence reaction. Light generated by this reaction is detected and analyzed by the Theranos System using a calibration function to determine the cut-off index (COI) values for the sample and controls. The results for the Positive and Negative controls must be within specified limits for a run to be considered valid

MATERIALS PROVIDED

Theranos Capillary Tubes and Nanotainer Tubes with K_2-EDTA (purple)

The Theranos Capillary Tubes and Nanotainer Tubes are non-sterile, single use only collection devices consisting of two Capillary Tubes paired with two Nanotainer Tubes that are capable of collecting, preserving, and transporting fingerstick whole blood samples for use with the Theranos HSV-1 IgG Assay. Each capillary is coated with di-potassium EDTA (K_2-EDTA) anticoagulant which is intended to preserve the specimen during transport and/or storage (there are no anticoagulants in the Nanotainer Tubes). A needle interfaces with each capillary to transport blood from each capillary into a dedicated Nanotainer Tube.

Theranos Cartridge (HSV-1 IgG Assay)

The Theranos HSV-1 IgG Assay reagents are assembled in a self-contained, ready-to-use pouched cartridge that is sufficient to run one test. The pouched cartridge should be removed from the refrigerator at least 10 minutes prior to use, but must be used within 24 hours after removal from the refrigerator. Any cartridge that has exceeded the 24 hour time limit should be discarded. The cartridge pouch should be kept sealed until it is needed to run a sample. The components of the cartridge are described in Table 1 below:

Table 1: Description of Materials Provided with the Theranos HSV-1 IgG Assay

Capture Surface	Coated with HSV-1 gG-1 recombinant antigen solution containing stabilizers and preservatives.
Detection Reagent (65 µL)	Mouse monoclonal antibody against human IgG, conjugated to alkaline phosphatase (AP) in a solution containing stabilizers and preservatives.
Positive Control (65 µL)	Liquid, human serum based, containing HSV-1 IgG.

Negative Control (65 µL)	Liquid, human serum based, containing no HSV-1 IgG.
Assay Diluent (160 µL)	Phosphate buffered saline (PBS).
Wash Buffer (65 µL)	Tris buffered saline (TBS) with detergent.
Chemiluminescent AP substrate reagent (65 µL)	Chemilumiscent substrate formulation for alkaline phosphatase.

Theranos TSPU Device

The Theranos TSPU Device performs automated sample processing and signal detection. Raw data collected are analyzed by the Theranos System under the oversight of the Theranos CLIA-certified laboratory. The Theranos TSPU is intended to be used only with Theranos assay cartridges.

MATERIALS REQUIRED BUT NOT PROVIDED

- Disposable gloves – gloves must be worn during the sample collection procedure. Gloves must be worn during the sample processing procedure.
- Disposable absorbent workplace cover – for use in the sample collection procedure, to cover the workplace area used for sample collection. For use in the sample processing procedure, to cover the clean, flat workplace surface where the cartridge and sample are handled for sample processing on the TSPU device.
- Antiseptic wipes – for use in the sample collection procedure, to cleanse the patient's fingerstick sample collection site.
- Disinfectant wipes – if required during sample collection procedure, following sample transfer from Capillary Tubes to Nanotainer Tubes, to wipe any excess blood from the outside of the Nanotainer Tubes.
- Sterile safety lancet – single use, disposable, for use in the sample collection procedure to create the fingerstick puncture site.
- Warming device – commercially available, single use, disposable, for use in the sample collection procedure. It is recommended that the patient's finger is warmed for 45 seconds using the warming device prior to the skin puncture.
- Sterile gauze – for use in the sample collection procedure, to wipe any excess blood from the patient's fingertip following sample collection.
- Biohazard bin – For use in the sample processing procedure, to dispose of the used cartridge following sample processing, for use following the sample collection procedure to dispose of single use articles in contact with blood.
- Sharps container – for use in the sample collection procedure, to dispose of the used safety lancet.
- K2-EDTA Tube – a standard K2-EDTA collection tube for venipuncture (if required).

THERANOS PATIENT SERVICE CENTERS – SPECIMEN COLLECTION AND PREPARATION

The Theranos HSV-1 IgG Assay is intended for use with K2-EDTA anticoagulated human plasma from fingerstick whole blood. Fingerstick whole blood samples should be collected with the Theranos Capillary Tubes and Nanotainer Tubes. This device is designed to collect 80µL of whole blood in each of a pair of Nanotainer Tubes (160µL of whole blood in the paired Tubes).

CLIA LABORATORY – SPECIMEN COLLECTION AND PREPARATION

The Theranos HSV-1 IgG Assay is intended for use with human serum from venous blood and K2-EDTA anticoagulated human plasma from venous and fingerstick whole blood. Venous blood should be collected aseptically using approved venipuncture techniques by qualified personnel. For the collection of fingerstick specimens, fingerstick whole blood samples should be collected with the Theranos Capillary Tubes and Nanotainer Tubes in accordance with the instructions provided. For the serum specimens collected by venipuncture, the blood should be allowed to clot, and the serum separated from the clot as soon as possible. Store and ship Nanotainer Tubes in controlled insulated and refrigerated containers specified by Theranos, following Nanotainer Tubes sample storage and shipment instructions as specified by Theranos. Store and ship venous samples in controlled insulated and refrigerated containers specified by Theranos, following venous sample storage and shipment instructions as specified by Theranos.

Upon receipt in the CLIA-certified laboratory, the fingerstick whole blood samples in the Nanotainer Tubes should be centrifuged at 1200g for 5 minutes within 48 hours of sample collection. The venous K2-EDTA anticoagulated whole blood samples should be centrifuged at 1300g for 10 minutes per standard vacutainer protocols.

The separated plasma from fingerstick specimens can remain at room temperature for no longer than 6 hours. If the assay will not be completed within 6 hours, refrigerate the sample at 2 to 8°C. If the assay will not be completed within 48 hours of storing at 2-8°C, the samples should be frozen at –20°C. Frozen specimens should be thawed and mixed well prior to use. A maximum of 3 freeze thaw cycles are allowed.

The minimum volume required from the sample in the collection device is approximately 30 µL of specimen (plasma or serum) (10 µL of specimen is used for the assay and approximately 20 µL of dead volume).

CAUTION: All specimens must be treated as potentially infectious material.

CLIA LABORATORY – THE HSV-1 IGG ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the following operating instructions:

1. Pouched "HSV-1 IgG Assay, CLIA lab location" cartridges should be stored at 4°C. They should be kept right-side-up as indicated by a sticker on the top side of each pouch.
2. **For fingerstick whole blood samples provided in the Capillary Tubes and Nanotainer Tubes:**
    a. Scan QR code on bottom of Nanotainer Tube into SampleID field of the tracking file.

b. Open cartridge pouch labelled "HSV-1 IgG Assay, CLIA lab location" and discard wrapper and desiccant in biohazard bin.

c. Scan QR code on side of cartridge into Barcode field of tracking file and enter the patient number into the PatientID field.

d. Orient the cartridge such that the barcode is on the right hand side and the Nanotainer Tube slot is on the upper right. The green arrow on the cartridge should point into the device.

e. Transfer 30µL of plasma using a manual pipette into the round vessel highlighted with a red ring near the center of the "Theranos HSV-1 IgG Assay, CLIA lab location" cartridge.

3. **For venous blood collected with a commercially available venous blood EDTA-anti-coagulated collection device or commercially available serum collection device:**
   a. Scan barcode on the sample container into SampleID field of the tracking file.
   b. Open cartridge pouch labelled "HSV-1 IgG Assay, CLIA lab location" and discard wrapper and desiccant in biohazard bin.
   c. Scan QR code on side of cartridge into Barcode field of tracking file and enter the patient number into the PatientID field.
   d. Orient the cartridge such that the barcode is on the right hand side and the Nanotainer Tube slot is on the upper right. The green arrow on the cartridge should point into the device.
   e. Transfer 30µL of plasma or serum using a manual pipette into the round vessel highlighted with a red ring near the center of the "Theranos HSV-1 IgG Assay, CLIA lab location" cartridge.
4. Prompts on the TSPU touch screen should be followed to insert the cartridge into the TSPU and to start the test run.
5. Once the TSPU application has initialized, the Home Screen will appear. Tap the Home Screen to begin.
6. Tapping on the Home Screen brings you to the Open Screen.
7. Touch the Open Button to open the cartridge door on the front of the TSPU. This will start a 90-second countdown timer.
8. If the cartridge is not inserted within the 90-second period, the door will close. If this happens, the screen will go back to the Open Screen (Step 6). Press the Open Button to reopen the cartridge door (Step 7).
9. Insert the cartridge so its green arrow points into the TSPU. Upon detection, the TSPU will pull the cartridge into the TSPU.
10. Once the cartridge is pulled into the TSPU, the screen will indicate that the processing is initializing.
11. Once the initialization is complete, the TSPU will automatically execute the protocol and the screen will display a circular indicator displaying the percentage of the total

time to execute the protocol in its center, and an estimated countdown of the time remaining to complete the protocol.

12. When the protocol is successfully completed, a "complete" message will appear briefly indicating that the process completed successfully, followed by a "Test complete" screen.
13. The final Eject Screen will indicate that the test has been completed and the cartridge may be ejected by pressing the button.
14. The door will open and the cartridge will be ejected. The door will remain open for up to 120 seconds at which time the TSPU retracts the cartridge and shuts the door to maintain the temperature within the TSPU. In that case, the screen will go back to the Eject Screen (Step 13).
15. The cartridge should be discarded into a biohazard bin.
16. The TSPU detects that the ejected cartridge is removed, and the cartridge door will close and navigate back to the Home Screen.

Once the cartridge is loaded into the TSPU the following steps are automatically implemented in sequence over approximately 78 minutes.

1. Sample plasma, negative control material and positive control material are mixed with sample diluent to yield sample diluent and diluted control samples in separate vessels.
2. During the first incubation step, the HSV-1 IgG antibodies present in the controls and sample bind an HSV-1 glycoprotein G (gG) recombinant antigen on a coated surface.
3. Following the first incubation step, unbound materials are removed with a wash cycle.
4. Then the detection reagent-AP conjugate is added and during the second incubation step, the detection reagent-AP conjugate reacts with the HSV-1 IgG antibodies already bound to the capture surface.
5. Following the second incubation, unbound materials are removed with a wash cycle.
6. The chemiluminescent substrate is added to the capture-analyte-detection complex during the third incubation step to initiate the chemiluminescence reaction.
7. Light generated by this reaction is detected and analyzed by the Theranos System using a calibration function to determine the cut-off index (COI) values for the sample.

QUALITY CONTROL (QC)

The Theranos HSV-1 IgG Assay cartridges are completely self-contained and ready-to-use, and include the use of on-board positive and negative controls. Each laboratory should follow applicable local laws, regulations and standard good laboratory practice to establish its own QC ranges and frequency of QC testing. The on-board positive and negative controls are purchased from commercial sources. These on-board control samples are run simultaneously with the assay each time a patient sample is run, processed in parallel with the patient samples, in exactly the same way as the patient sample is processed.

Expected Control Results

Control Type	Expected result
Positive control: Liquid (65 µL), human serum based, containing HSV-1 IgG	Positive
Negative control: Liquid (65 µL), human serum based, containing no HSV-1 IgG.	Negative

The output from these positive and negative control runs are analyzed by the Theranos System for any performance deficiencies in the system in real time. Calibration functions consist of pass/fail limits, for both the positive control and the negative control. If the control value lies outside the pass limit, the run is considered to be invalid. If either control fail, or if it is out of range, the test result will be INVALID as described in Table 2 below.

INTERPRETATION OF RESULTS

The result returned by the Theranos HSV-1 IgG test automatically factors in the validity of on-board controls. The user obtains a result with one of the following possibilities:

Table 2: Interpretation of results

Result	Interpretation	Follow up testing recommendation
POSITIVE	HSV-1 specific IgG antibodies detected	None
NEGATIVE	HSV-1 specific IgG antibodies not detected	None
EQUIVOCAL	HSV-1 specific IgG antibody status equivocal	CLIA Laboratory: Samples should be re-tested. If on re-test, the sample is still equivocal, then a second sample should be drawn within 4-6 weeks and tested.
INVALID	Invalid result	The test result should be discarded and the test should be re-run.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Precision –CLIA Laboratory Model, Venous Serum

   A study for estimating the precision of the Theranos HSV-1 IgG Assay for venous serum samples in a CLIA Laboratory model was performed by testing a panel of 6 serum samples spanning the analytical range [negative (A), high negative (B), equivocal (C), low positive (D), moderate positive (E), and positive (F)]. The precision study was conducted at one site with thirty five (35) TSPU devices, three (3) lots of cartridges and sixteen (16) operators in total. The study duration was 13 days in total. Details of the study design for different samples are presented in Table 3 below.

Table 3: Design of Precision Study: Numbers of Replicates, Devices, Days and Operators

Panel Member	Valid Replicates				No. of Devices	No. of Days	No. of Operators	No. of Invalid Replicates
	Total	Lot 1	Lot 2	Lot 3				
A (Negative)	91	26	38	27	35	7	14	3
B (High Neg.)	88	24	37	27	28	7	14	2
C (Equivocal)	78	27	44	8	35	8	16	3
D (Low Pos.)	80	25	27	28	11	2	4	4
E (Mod. Pos.)	64	25	13	26	13	2	6	1
F (Positive)	69	25	19	25	15	2	4	3

Results of the precision study are presented in Table 4.

Table 4: Summary of Precision Study Results

Panel Member	Mean (COI)		Repeatability (same device, same lot)	Between-device	Between-lot	Precision (same device, different lot)	Precision (different device, same lot)	Precision (different device, different lot)
A (Negative)	0.425	SD	0.049	0.007	0.000	0.049	0.049	0.049
		%CV	11.5%	1.6%	0%	11.5%	11.6%	**11.6%**
B (High Neg.)	0.648	SD	0.086	0.011	0.029	0.091	0.087	0.092
		%CV	13.3%	1.7%	4.5%	14.1%	13.4%	**14.2%**
C (Equivocal)	1.016	SD	0.093	0.062	0.065	0.113	0.112	0.129
		%CV	9.1%	6.1%	6.4%	11.1%	11.0%	**12.7%**
D (Low Pos.)	1.727	SD	0.208	0.098	0.013	0.208	0.230	0.230
		%CV	12.0%	5.7%	0.8%	12.0%	13.3%	**13.3%**
E (Mod. Pos.)	3.809	SD	0.305	0.276	0.108	0.324	0.411	0.425
		%CV	8.0%	7.3%	2.8%	8.5%	10.8%	**11.2%**
F (Positive)	8.996	SD	0.807	0.437	0.000	0.807	0.918	0.918
		%CV	9.0%	4.9%	0.0%	9.0%	10.2%	**10.2%**

Table 5 presents percent of invalid results and percents of negative, equivocal and positive among valid results for each sample.

Table 5: Percent of Invalid Results and Percents of Negative, Equivocal and Positive among Valid Results

Panel Member	Mean (COI)	Number of Replicates	Percent of Invalid	Percent of Negative among Valid	Percent of Equivocal among Valid	Percent of Positive among Valid
A (Negative)	0.425	94	3.2% (3/94)	100% (91/91)		
B (High Neg.)	0.648	90	2.2% (2/90)	100% (88/88)		
C (Equivocal)	1.016	81	3.7% (3/81)	17.9% (14/78)	60.3% (47/78)	21.8% (17/78)
D (Low Pos.)	1.727	84	4.8% (4/84)			100% (80/80)
E (Mod. Pos.)	3.809	65	1.5% (1/65)			100% (64/64)
F (Positive)	8.996	72	4.2% (3/72)			100% (69/69)

The results of the study demonstrate that the precision of the Theranos HSV-1 IgG Assay (including different TSPU devices, different lots of cartridges, and different operators) when performed in a CLIA Laboratory was in the range 10.2% to 14.2%.

Precision –CLIA Laboratory Model, Fingerstick Whole Blood

A study for estimating the precision of the Theranos HSV-1 IgG Assay for fingerstick whole blood samples in a CLIA Laboratory model was performed by testing a panel of 3 fingerstick plasma samples spanning the analytical range [high negative (P), equivocal (Q), moderate positive (R)]. The precision study was conducted at one site with thirty-six (36) TSPU devices, three (3) lots of cartridges and nine (9) operators in total. The study duration was 4 days in total. Details of the study design for different samples are presented in Table 6 below.

Table 6: Design of Precision Study: Numbers of Replicates, Devices, Days and Operators

Panel Member	Valid Replicates				No. of Devices	No. of Days	No. of Operators	No. of Invalid Replicates
	Total	Lot 1	Lot 2	Lot 3				
P (High Neg.)	168	56	56	56	30	4	9	3*
Q (Equivocal)	168	56	56	56	29	4	9	2*
R (Mod. Pos.)	168	56	56	56	27	4	9	2*

*All invalid replicates were repeated.

Results of the precision study are presented in Table 7.

Table 7: Summary of Precision Study Results

Panel Member	Mean (COI)		Repeatability (same device, Same Lot)	Between-Device	Between-Lot	Precision (same device, different lot)	Precision (different device, same lot)	Precision (different device, different lot)
P (High Neg.)	0.888	SD	0.083	0.006	0.050	0.096	0.083	0.097
		%CV	9.3%	0.7%	5.6%	10.9%	9.3%	**10.9%**
Q (Equivocal)	1.047	SD	0.094	0.025	0.069	0.117	0.098	0.119
		%CV	9.0%	2.4%	6.6%	11.1%	9.3%	**11.4%**
R (Mod. Pos.)	3.241	SD	0.342	0.122	0.157	0.377	0.363	0.396
		%CV	10.6%	3.8%	4.9%	11.6%	11.2%	**12.2%**

Table 8 presents percent of invalid results and percents of negative, equivocal and positive among valid results for each sample.

Table 8: Percents of Positive, Equivocal, Negative and Invalid Results

Panel Member	Mean (COI)	Number of Replicates	Percent of Invalid	Percent of Negative among Valid	Percent of Equivocal among Valid	Percent of Positive among Valid
P (High Neg.)	0.888	171	1.8% (3/171)	58.3% (98/168)	40.5% (68/168)	1.2% (2/168)
Q (Equivocal)	1.047	170	1.2% (2/170)	6.5% (11/168)	63.1% (106/168)	30.4% (51/168)
R (Mod. Pos.)	1.016	170	1.2% (2/170)			100% (168/168)

The results of the study demonstrate that the precision of the Theranos HSV-1 IgG Assay (including different TSPU devices, different lots of cartridges, and different operators) when performed in a CLIA Laboratory was in the range from 10.9% to 12.2%.

Reproducibility

A study designed to process multiple fingerstick whole blood samples from individual subjects was performed to evaluate the reproducibility of the Theranos HSV-1 IgG Assay when used with Theranos Capillary Tubes and Nanotainer Tubes. The study was conducted at 3 collection sites with 10 subjects at each site. From each of 30 subjects, 9 Capillary Tubes and Nanotainer Tubes from 3 manufacturing lots (i.e. 3 Capillary Tubes and Nanotainer Tubes per lot) and 2 serum separator tubes (SSTs) were collected. Each subject had the following measurements:

- Each of the 9 Capillary Tubes and Nanotainer Tubes was tested. These data were used for the evaluation of Between-Capillary Tubes and Nanotainer Tubes imprecision, Between-lot imprecision and Total imprecision that includes Between-Capillary Tubes and Nanotainer Tubes and Between-lot imprecisions.
- One Nanotainer Tube (from one of the 3rd lot of Capillary Tubes and Nanotainer Tubes for each subject) was tested in duplicate via recovering a sample from one Capillary Tubes and Nanotainer Tubes device and transferring a sample to another Capillary Tubes and Nanotainer Tubes device. These data were used for evaluation of Within-Capillary Tubes and Nanotainer Tubes imprecision.
- Each of the 2 SSTs was tested. These data were used for evaluation of Between-SST imprecision.

For samples with mean COI value at the baseline ≥0.5, percent differences were calculated and for samples with mean COI value at the baseline <0.5, differences were calculated. Table 9 summarizes the results of the precision study broken down by collection site and by high or low COI subjects; the variability metrics are averaged across all subjects within the site.

Table 9: Summary of Results of the Reproducibility Study

Collection Site	Subjects	Capillary Tubes and Nanotainer Tubes				SST
		Within-Capillary Tubes and Nanotainer Tubes (%CV or SD)	Between-Capillary Tubes and Nanotainer Tubes (%CV or SD)	Between-Lot (%CV or SD)	Total (%CV or SD)	Between-SST (%CV or SD)
1	6 subjects with COI values 1.4 – 13.5	%CV=6.0%	%CV = 9.0%	%CV = 6.8%	%CV=12.6%	%CV=9.6%
	4 subjects with COI values 0.03-0.28	SD=0.008	SD = 0.015	SD = 0.016	SD = 0.024	SD=0.11
2	7 subjects with COI values 1.6 – 16.8	%CV=8.2%	%CV=9.2%	%CV=3.2%	%CV=10.8%	%CV=12.5%
	3 subjects with COI values 0.07-0.19	SD=0.009	SD=0.011	SD=0.008	SD=0.015	SD=0.019
3	5 subjects with COI values 4.5 – 14.3	%CV=8.2%	%CV=8.1%	%CV=6.0%	%CV=11%	%CV=12.4%
	5 subjects with COI values 0.02-0.32	SD=0.08	SD=0.019	SD=0.013	SD=0.025	SD=0.021
Combined	18 subjects with COI values 1.4-16.8	%CV=7.5%	%CV=8.8%	%CV=5.2%	%CV=11.4%	%CV=11.5%
	12 subjects with COI values 0.02-0.32	SD=0.008	SD=0.015	SD=0.013	SD=0.022	SD=0.017

- Within-Capillary Tubes and Nanotainer Tubes imprecision was %CV=7.5% for aggregated subjects with a mean COI ≥0.5 and SD=0.008 for aggregated subjects with mean COI <0.5.

- Total imprecision including Between-Capillary Tubes and Nanotainer Tubes and Between-lot imprecisions was %CV= 11.4% for aggregated subjects with a mean COI ≥0.5 and SD=0.022 for aggregated subjects with a mean COI<0.5.

- Between- serum separator tubes imprecision was %CV=11.5% for aggregated subjects with a mean COI ≥0.5 and SD=0.017 for aggregated subjects with a mean COI <0.5.

b. Linearity/assay reportable range:

Not Applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls: The two on-board controls used in the test are from commercially available sources. Acceptable limits for control measurements were established by averaging 813 runs. Any control measurement outside the acceptable range invalidates the cartridge run.

Calibrators: Calibrators for factory calibration are formulated using commercially available calibrators and an in-house standard to obtain three calibrator levels at COI values of 0.9, 1.1 and 2.0. Calibration of Theranos HSV-1 IgG assay is tied to a particular lot of reagents, and a particular lot of cartridges. The cut-off is set for the lot of cartridges based on the Relative Light Units value of the cut-off calibrator. The other calibrators are used to verify the modulation of the assay. The modulation should be within specified conditions. If these conditions are not satisfied, the lot is considered to not have sufficient modulation and is disqualified.

<u>Analyte Stability</u>

An analyte stability study was performed to characterize the stability of HSV-1 IgG in clinical matrices as measured by the Theranos HSV-1 IgG Assay under different sample storage conditions and time periods as shown in Table 10.

Table 10: Summary of Analyte Storage Conditions and Durations for Different Sample Types and Matrices Claimed for Theranos HSV-1 IgG Assay

Condition	Venous Serum	Venous K_2-EDTA Plasma	Fingerstick K_2-EDTA Plasma	Fingerstick K_2-EDTA Whole Blood
Stored at 2-8°C	48 hr	48 hr	48 hr	48 hr
Stored at room temperature (20-25°C)	6 hr	6 hr	6 hr	6 hr

Condition	**Venous Serum**	**Venous K_2-EDTA Plasma**	**Fingerstick K_2-EDTA Plasma**	**Fingerstick K_2-EDTA Whole Blood**
Stored at -20°C	1 week	1 week	1 week	N/A
Freeze/thaw cycles	3	3	3	N/A

Within 2 hours after collection, one aliquot of each sample type or matrix was tested with the Theranos HSV-1 IgG Assay in duplicate, to establish the value at baseline. The samples were stored in Nanotainer Tubes under the appropriate conditions. Comparison of an average of two replicates at the predetermined time points with the average of two replicates at baseline was performed. For samples with a mean COI value at the baseline ≥0.5, percent differences were calculated and for samples with a mean COI value at the baseline <0.5, differences were calculated.

Acceptance criteria were as follows: i) a difference averaged over all samples with a baseline mean COI value ≥0.5 must be less than ±10% and a difference averaged over all samples with a baseline COI mean <0.5 must be less than 0.02 and ii) for each sample, an observed difference must be less than 15% for the samples with a baseline mean COI value ≥0.5 and must be less than 0.08 for the samples with a baseline mean COI value <0.5 (the range of differences expected if there is no effect of storage on the HSV-1 IgG analyte).

Table 11: Summary of Mean Absolute Difference Measures for all Storage Conditions and Sample Types or Matrices

	Sample Type and Matrix	Samples with a baseline COI < 0.5		Samples with a baseline COI > 0.5	
		Difference averaged over all samples	The largest observed difference among samples	Percent difference averaged over all samples	The largest observed percent difference among samples
Stored at 2-8C, 48 hrs	Venous serum	0.006	0.006	1.0%	13.6%
	Venous K2-EDTA plasma	-0.007	-0.007	2.3%	13.3%
	Fingerstick K2-EDTA plasma from whole blood	0.003	0.003	-0.4%	13.9%
Stored at -20, 1 week	Venous serum	0.008	0.015	0.8%	13.3%
	Venous K2-EDTA plasma	0.003	0.005	-1.0%	12.7%
	Fingerstick K2-EDTA plasma from whole blood	0.001	0.008	1.8%	-13.9%
Freeze thaw cycles, n=3	Venous serum	0.007	0.021	-0.1%	13.6%
	Venous K2-EDTA plasma	0.021	0.037	-1.7%	-13.4%
	Fingerstick K2-EDTA plasma from whole blood	0.006	0.022	-1.0%7	13.6%
Stored at room temp, 6 hrs	Venous serum	-0.001	-0.011	-3.2%	-11.9%
	Venous K2-EDTA plasma	0.002	0.022	0.1%	13.7%
	Fingerstick K2-EDTA plasma from whole blood	-0.004	-0.026	1.1%	13.9%

d. Detection limit:

Not Applicable

e. Analytical specificity:

Interfering Substances

A study was designed and performed (in accordance with CLSI EP07-A2) to evaluate the performance of the Theranos HSV-1 IgG Assay in the presence of potentially interfering substances to assess the impact of these endogenous substances and commonly used drugs on the performance of the Theranos HSV-1 IgG Assay. Interferents were tested with three serum samples (negative (mean COI 0.024), high negative (mean COI 0.77) and low positive (mean COI 1.52)) that were contrived by using a high positive sample and diluting it with pooled negative serum. Samples were spiked with the interferent at levels shown in Table 12. Each serum pool was tested in duplicate.

For the low positive and the high negative pools, the acceptance criteria were a mean recovery within +/- 20% of the value of the unspiked sample (i.e., in the absence of the potential interferent or drug). All low positive and high negative samples showed a signal change of less than 15% for all interfering substances. All positive samples remained positive and all negative samples remained negative upon spiking of drug or other interferents. For the negative pool, the acceptance criterion was a deviation of less than 0.02 COI. All negative samples showed a mean deviation of ≤0.02 COI, except Intralipid. Intralipid spikes did not show any effect on recovery for near cut-off samples, high negative and low positive samples.

Table 12: Summary of Interfering Substances Studies: Endogenous Interferents and Drug Interferents

		Negative Pool		High Negative Pool		Low Positive Pool	
Interferent	Level	Mean COI	ΔCOI	Mean COI	% Recovery	Mean COI	% Recovery
Hemoglobin	1000 mg/dL	0.025	0.00	0.69	90	1.71	113
Bilirubin	20 mg/dL	0.024	0.00	0.68	88	1.61	106
Intralipid	2000 mg/dL	0.053	0.03	0.81	105	1.60	105
Acetylcysteine	150 mg/L	0.019	-0.004	0.68	88	1.40	92
Ampicillin-Na	1000 mg/L	0.025	0.001	0.76	99	1.44	95
Ascorbic acid	300 mg/L	0.027	0.003	0.75	97	1.67	110
Ca-Dobesilate	200 mg/L	0.027	0.004	0.70	91	1.51	99
Cyclosporine	5 mg/L	0.031	0.008	0.74	97	1.53	101
Cefoxitin	2500 mg/L	0.027	0.003	0.74	97	1.52	100

		Negative Pool		High Negative Pool		Low Positive Pool	
Interferent	Level	Mean COI	ΔCOI	Mean COI	% Recovery	Mean COI	% Recovery
Heparin	5000U	0.020	-0.003	0.80	103	1.52	100
Levodopa	20 mg/L	0.030	0.006	0.68	88	1.42	94
Methyldopa+1.5h20	20 mg/L	0.024	0.000	0.74	97	1.37	90
Metronidazole	200 mg/L	0.039	0.016	0.74	96	1.38	91
Phenylbutazone	400 mg/L	0.021	-0.002	0.74	96	1.42	94
Doxycycline	50 mg/L	0.024	0.000	0.71	92	1.35	89
Acetylsalicylic acid	1000 mg/L	0.026	0.002	0.75	97	1.37	90
Rifampicin	60 mg/L	0.014	-0.009	0.69	90	1.35	89
Acetaminophen	200 mg/L	0.034	0.010	0.64	83	1.68	111
Control		0.024	0.000	0.77	100	1.52	100

Cross-reactivity

A study was performed to evaluate the performance of the Theranos HSV-1 IgG Assay in the presence of IgG antibodies against twenty-one (21) infectious agents defined as potential cross-reactants in the FDA guidance on HSV serological assays. Banked serum samples confirmed positive for IgG against the infectious agents of interest were acquired from commercial vendors. At least three (3) samples, independently confirmed as positive for that agent and negative for HSV-1 IgG on the reference method, were tested on the Theranos HSV-1 IgG Assay in order to rule out cross-reactivity of the Theranos HSV-1 IgG Assay with IgG against a potential cross reactant. The results of this study are displayed in Table 13 below.

Table 13: Summary of Cross-reactivity Study on Theranos HSV-1 IgG Assay

Organism/Condition	No.	Reference HSV-1 Assay	Theranos HSV-1 Positive	Theranos HSV-1 Negative	Theranos HSV-1 Equivocal
Epstein Barr Virus (IgG)	6	Negative	0	6	0
Epstein Barr Virus (IgM)	1	Negative	0	1	0
HPV	4	Negative	0	4	0

Organism/Condition	**No.**	**Reference HSV-1 Assay**	**Theranos HSV-1 Positive**	**Theranos HSV-1 Negative**	**Theranos HSV-1 Equivocal**
Rubella (IgG)	13	Negative	0	13	0
HSV-2 (IgG)	40	Negative	0	40	0
HAMA samples	4	Negative	0	4	0
Treponema pallidum	8	Negative	0	7	1*
Rheumatoid Factor (RF)	8	Negative	1**	7	0
Anti-nuclear antibody (ANA)	8	Negative	0	8	0
Sjogren's Syndrome	3	Negative	0	3	0
CMV (IgG)	5	Negative	0	5	0
CMV (IgM)	2	Negative	0	2	0
Chlamydia trachomatis (IgG)	10	Negative	0	10	0
HCV (IgG)	3	Negative	0	3	0
HBsAg	3	Negative	0	3	0
VZV IgG	5	Negative	0	5	0
Measles IgG	5	Negative	0	5	0
HIV-1 (IgG)	4	Negative	0	4	0
Toxoplasma IgG	4	Negative	0	4	0
Candida albicans Ag	3	Negative	0	3	0
Systemic Lupus	3	Negative	0	3	0

*Systematic cross-reactivity ruled out (7/8 samples in same category tested negative)

**Confirmed as positive upon retest; systematic cross-reactivity ruled out (7/8 samples in same category tested negative)

f. Assay cut-off:

A study was performed to establish the cut-off and the limits of the equivocal zone for the Theranos HSV-1 IgG Assay using 192 serum samples. Then 120 independent serum samples were analyzed to validate the established cut-off. The calibrators were assigned COI values based on the established assay cut-off, the cut-off for positive results (COI of

1.1) and the cut-off for negative results (COI of 0.9). The results of the cut-off validation study are displayed in Table 14 below.

Table 14: Performance of Selected Cut-off on Independent Sample Set

Agreement Classification	Percent Agreement	95% Confidence Interval
NPA	96.0% (47/49)	86.3-98.9
PPA	97.1% (69/71)	90.3-99.2

2. Comparison studies:

a. Method comparison with predicate device:

Fingerstick Plasma – CLIA Laboratory Model

To demonstrate the performance of the Theranos HSV-1 IgG Assay for fingerstick whole blood samples were collected at 3 Theranos Patient Service Centers (TPSCs) and processed at the CLIA-certified laboratory.

At each site, fingerstick whole blood samples were collected into a pair of Theranos Capillary Tubes and Nanotainer Tubes, and venous samples were collected into serum tubes from 20, 16 and 25 adult subjects at three collection sites.

Samples were shipped refrigerated to the Theranos CLIA-certified laboratory in Palo Alto, CA. Upon receipt, fingerstick whole blood samples in the Nanotainer Tubes were centrifuged at 1200g for 5 minutes. Plasma was extracted and processed and analyzed on the Theranos System. All samples were processed or frozen as plasma within 48 hours of draw. The venous samples were processed into serum for testing with the reference method (FOCUS HerpeSelect Immunoblot).

A summary of the performance information is shown in the following Table 15.

Table 15: Summary of Method Comparison for Samples Collected at 3 Theranos Patient Service Centers

		Reference Result	
		POS	NEG
Theranos Result	POS	38	0
	NEG	1	22

	Point Estimate	95% Confidence Interval
Sensitivity	97.4% (38/39)	86.8 – 99.6
Specificity	100% (22/22)	85.1 – 100

b. Matrix comparison:

The effect of anticoagulants and different sample types (fingerstick and venous) on the performance of the Theranos HSV-1 IgG Assay was determined by comparing matched venous serum, venous K2-EDTA plasma, and fingerstick K2-EDTA plasma samples from 70 donors. Forty-three matched sample sets were contrived to have analyte values close to the cut-off. The acceptance criterion was a recovery of positive plasma samples within ± 20% of the corresponding serum reference value (serum drawn into primary tubes without gel). For negative samples, the acceptance criteria was a difference of <= 0.02 COI from the corresponding serum value. All anticoagulant–treated plasma samples met this criterion. Weighted Deming regression was performed. The slope and an intercept of the regression line and their 95% confidence intervals along with correlation coefficients are shown in Table 16 and a graphical depiction is shown in Figure 1.

Table 16: Summary of Weighted Deming Regression Analysis Performed on Matrix Equivalency Data for Venous Plasma and Fingerstick Plasma Samples.

Sample type/Matrix	Correlation coefficient	Slope	95% confidence interval on slope	Intercept	95% confidence interval on intercept
Venous plasma	0.992	0.993	[0.967, 1.019]	0.000	[-0.003, 0.003]
Fingerstick plasma	0.995	1.009	[0.973, 1.044]	-0.003	[-0.006, -0.001]

Figure 1: Regression Analysis for Matrix Equivalency Study

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

b. Clinical specificity:

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Clinical Performance in the Intended Use Populations (CLIA Laboratory Model)

A clinical study was conducted to characterize the performance of the Theranos HSV-1 IgG Assay in the Theranos CLIA-certified Laboratory in comparison to the FOCUS HerpeSelect Immunoblot (as the reference method for performance analysis).

Prospectively collected, archived venous serum samples collected from pregnant women and sexually active adults (18 years and older) who had a prescription for a HSV-1 IgG test. Samples were obtained from multiple specimen sources covering 10 US states and Mexico.

The equivocal results on the Focus HerpeSelect Immunoblot (that repeatedly tested equivocal) were resolved using a validated western blot reference test (University of Washington, Seattle) as per the instructions of the package insert for the reference method. Ten samples from the sexually active adult sub-population tested initially equivocal on the Focus HerpeSelect Immunoblot and were resolved by the University of Washington western blot as 2 negatives and 7 positives. One sample was not resolved. There were no samples in the sexually active adult sub-population that returned an invalid result.

In the pregnant women sub-population, 8 samples tested initially equivocal on the Focus HerpeSelect immunoblot. Of these, 4 samples could not be resolved by the University of Washington western blot due to insufficient volume availability. Of the remaining 4 samples, 1 (one) were resolved as negative and 3 as positive. There were 3 samples that returned an invalid result on the Theranos HSV-1 IgG test. These samples were rerun and resulted in valid results included in the analysis. The clinical performance information for the sexually active adults and pregnant women population is presented in Tables 17 and 18 below:

Table 17: Summary of Theranos HSV-1 IgG Test Performance with Sexually Active Adult Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	137	0	2	139
	Equivocal	1	0	1	2
	Negative	5	1	113	119
	Total	143	1	116	***260***

	Point Estimate	95% Confidence Interval
Sensitivity	95.1% (137/144)	90.3-97.6
Specificity	97.4% (113/116)	92.7-99.1

Table 18: Summary of Theranos HSV-1 IgG Test Performance with Pregnant Women Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	188	1	4	193
	Equivocal	0	1	0	1
	Negative	2	2	100	104
	Total	190	4	104	***298***

	Point Estimate	95% Confidence Interval
Sensitivity	97.9% (188/192)	94.8-99.2
Specificity	95.2% (100/105)	89.3-98.0

CDC Panel Testing:

The objective of this study was to demonstrate agreement of the Theranos HSV-1 IgG Assay with the CDC panel. A panel of well characterized serum samples (n=100) was obtained from the U.S. Centers for Disease Control and Prevention (CDC). The CDC sample panel was tested with the HSV-1 IgG Assay and the results obtained by Theranos were sent to the CDC for confirmation. The panel consisted of 54 positives and 46 negatives. The Theranos HSV-1 IgG Assay demonstrated 100% agreement with the results provided by the CDC.

Low Prevalence Population

Serum samples were collected from a low prevalence population: individuals who are not sexually active, and without a recent or current sexually transmitted disease (Hepatitis, Syphilis, HIV, HPV, Trichomonas, Chlamydia, and Gonorrhoeae) as determined in an interview. Performance of the assay on this population is summarized in Table 19. The Samples were obtained from multiple specimen sources covering 10 US states and Mexico.

Table 19: Summary of Theranos HSV-1 IgG Assay Performance with Low Prevalence Population

		Reference method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	32	0	0	32
	Equivocal	0	0	0	0
	Negative	0	1	49	50
	Total	32	1	49	***82***

	Point Estimate	95% Confidence Interval
Sensitivity	97.0% (32/33)	84.7-99.5
Specificity	100% (49/49)	92.7-100

4. Clinical cut-off**:**

Not Applicable

5. Expected values/Reference range:

Expected Values

The Theranos HSV-1 IgG Assay was used to evaluate the prevalence of HSV-1 IgG antibodies in individuals for whom an HSV-1 IgG test was ordered by a physician including pregnant women. The study populations for the Theranos HSV-1 IgG Assay consisted of a total of 558 subjects, with 260 sexually active adults and 298 individuals identified as pregnant women. The result for 1 out of the 558 subjects is not reported, as indicated in Table 20 (1 subject), giving a total of 557 subjects. The data for the intended use population (557 specimens) have been summarized according to age group in decades, gender, number of reactive results, and number of non-reactive results. The data for the intended use population have been summarized in Table 20 (259 specimens from sexually active adult subjects) and Table 21 (298 specimens from pregnant subjects).

Table 20: Expected Results for Theranos HSV-1 IgG Assay in Adult Subjects

Age Range	Gender	Reactive N/Total (%)	Equivocal N/Total (%)	Non-Reactive N/Total (%)
16 to 19	Male	0/0 (0)	0/0 (0)	0/0 (0)
16 to 19	Female	1/4 (25)	0/4 (0)	3/4 (75)
20 to 29	Male	8/18 (44.4)	1/18 (5.6)	9/18 (50)
20 to 29	Female	29/73 (39.7)	0/73 (0)	44/73 (60.3)
30 to 39	Male	5/10 (50)	0/10 (0)	5/10 (50)
30 to 39	Female	33/62 (53.2)	0/62 (0)	29/62 (46.8)
40 to 49	Male	5/10 (50)	0/10 (0)	5/10 (50)
40 to 49	Female	16/27 (59.3)	0/27 (0)	11/27 (40.7)
50 to 59	Male	17/20 (85)	0/20 (0)	3/20 (15)
50 to 59	Female	9/11 (81.8)	0/11 (0)	2/11 (18.2)
60 to 69	Male	5/6 (83.3)	0/6 (0)	1/6 (16.7)
60 to 69	Female	5/10 (50)	1/10 (10)	4/10 (40)
70 to 79	Male	3/4 (75)	0/4 (0)	1/4 (25)
70 to 79	Female	1/3 (33.3)	0/3 (0)	2/3 (66.7)
80 to 89	Male	0/0 (0)	0/0 (0)	0/0 (0)
80 to 89	Female	1/1 (100)	0/1 (0)	0/1 (0)
Total*		138/259 (53.3)	2/259 (0.8)	119/259 (45.9)

*1 sample not reported since age information was not available

Table 21: Expected Results for Theranos HSV-1 IgG Assay in Pregnant Subjects

Age Range	Gender	Reactive N/Total (%)	Equivocal N/Total (%)	Non-Reactive N/Total (%)
18 to 19	Female	13/13 (100)	0/13 (0)	0/13 (0)
20 to 29	Female	114/175 (65.1)	1/175 (0.6)	60/175 (34.3)
30 to 39	Female	61/104 (58.7)	0/104 (0)	43/104 (41.3)
40 to 49	Female	5/6 (83.3)	0/6 (0)	1/6 (16.7)
Total		193/298(65)	1/298 (0.3)	104/298 (35)

The hypothetical positive and negative predictive values (PPV, NPV) for the two intended use populations are shown in Table 22. The calculations are based on the specificity and sensitivity values for the Theranos HSV-1 IgG Assay determined in the clinical study;

1. Specificity of 97.4% and Sensitivity of 95.1% in sexually active adults
2. Specificity of 95.2% and Sensitivity of 97.4% in pregnant women

Table 22: Hypothetical Predictive Values

Prevalence (%)	Sexually Active Adults		Pregnant Women	
	PPV (%)	NPV (%)	PPV (%)	NPV (%)
50	93.8	92.6	92.1	91.7
45	93.2	93.2	91.3	92.4
40	92.4	93.8	90.3	93.0
35	91.4	94.2	89.1	93.5
30	90.1	94.6	87.5	94.0
25	88.3	94.9	85.3	94.3
20	85.8	95.2	82.3	94.7
15	82.0	95.5	77.7	95.0
10	75.2	95.7	69.9	95.2
5	60.2	96.0	53.8	95.5

N. Instrument Name:

Theranos Sample Processing Unit (TSPU)

Theranos Laboratory Automation System (TLAS)

O. System Descriptions:

1. Modes of Operation:

Does the applicant’s device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes _____X___ or No ________

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes ________ or No __X______

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No ________

3. Specimen Identification:

Sample identification is manually entered.

4. Specimen Sampling and Handling:

Each specimen is manually loaded into an assay cartridge.

5. Calibration:

The TSPU is calibrated by the manufacturer.

6. Quality Control:

Specific assay controls are included within each assay cartridge.

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

Q. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

EXHIBIT 7

To: Daniel Young[dyoung@theranos.com]
From: Sunny Balwani
Sent: Wed 7/15/2015 9:38:47 PM
Subject: FW: CW150009 CLIA Waiver Granted Notification
CW150009.pdf

From: Brad Arington
Sent: Wednesday, July 15, 2015 2:37 PM
To: Elizabeth Holmes; Sunny Balwani; Heather King
Subject: FW: CW150009 CLIA Waiver Granted Notification

Just came in.

Brad Arington
Theranos, Inc.
Associate Director, Regulatory
Direct: (650) 856-7304
Cell: [redacted]

From: Tobin, Peter [mailto:Peter.Tobin@fda.hhs.gov]
Sent: Wednesday, July 15, 2015 2:34 PM
To: Brad Arington
Cc: El Mubarak, Haja Sittana; Lovell, Stephen; Scherf, Uwe; Gutierrez, Alberto; CDRH CLIA Coordinator; official-CW150009@doc.fda.gov
Subject: CW150009 CLIA Waiver Granted Notification

Dear Mr. Brad Arington

Please find attached a CLIA Waiver Granted Notification regarding CW150009.

If you have any questions, please contact the lead reviewer, Haja Sittan El Mubarak.

Kind regards,

Peter

Peter Tobin, Ph.D.
Division of Program Operations and Management
Office of In Vitro Diagnostics and Radiological Health
FDA Center for Devices and Radiological Health
10903 New Hampshire Avenue
Building 66, Room 4529
Silver Spring, MD 20993
[redacted]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center - WO66-G609
10903 New Hampshire Ave.
Silver Spring, MD 20993-0002

July 15, 2015

Brad Arington, Associate Director, Regulatory
THERANOS, INC.
1701 PAGE MILL ROAD
PALO ALTO, CA 94304 US

Re: CW150009
CLIA Parent(s): k143236
Applicant: THERANOS, INC.
Device: Theranos Herpes Simplex Virus-1(HSV-1) IgG Assay
Dated: June 29, 2015
Received: June 30, 2015
CLIA Effective Date: July 15, 2015

Waiver Granted Notification

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your application for waived status under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations. We are pleased to inform you that your test system(s) as identified below is waived:

Test System/Analyte(s): (SEE ATTACHMENT)

Waived status is applicable to test systems and their instructions approved or cleared by the FDA. We recommend that the test system instructions include a statement that the test system is waived under CLIA. Any modification to the test system including test system instructions or a change in the test system name must be submitted to the FDA for the evaluation of waiver. If you change the test system name or your company's name or if a distributor's name replaces your name, you must request another categorization by sending in the revised labeling along with a letter to FDA referencing the document number above.

This complexity categorization is effective as of the date of this notification and will be reported on FDA's home page http://www.fda.gov/cdrh/clia . This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. FDA reserves the right to re-evaluate and re-categorize this test based upon additional information received.

If you have any questions regarding this complexity categorization, please contact Stephen Lovell at 301-796-6968.

Sincerely yours,

Alberto Gutierrez, Ph.D.
Director
Office of *In Vitro* Diagnostics and
Radiological Health
Center for Devices and Radiological Health

ATTACHMENT

Parent Number : k143236

Test System : Theranos anti-HSV-1 IgG Assay {Fingerstick Whole Blood Only}
Analyte : Herpes simplex I and/or II antibodies
Complexity : WAIVED

EXHIBIT 8

To: Hojvat, Sally A [redacted] Hobson, John (Peyton)
[redacted]
From: Elizabeth Holmes
Sent: Wed 10/23/2013 8:03:12 PM
Importance: Normal
Received: Wed 10/23/2013 8:03:00 PM
Assay Testing Summary in CLIA Lab_Wellness Centers_Theranos_Confidential.pdf
Planned Assay Testing Summary in CLIA Lab_Wellness Centers_Theranos_Confidential.pdf

TRADE SECRET/CONFIDENTIAL COMMERCIAL INFORMATION EXEMPT FROM DISCLOSURE UNDER THE FREEDOM OF INFORMATION ACT

Dear Sally and Peyton,

In follow up to our conversations on which assays run on Theranos TSPUs in Theranos' CLIA lab during Phase I of Theranos' operations (as described in the diagrams previously sent), please find two documents attached to this email.

The first document lists all tests that have been run on samples collected from patients in Theranos Wellness Centers since the Wellness Centers began operation in September.
For your reference, some samples collected in Theranos Wellness Centers to date have been collected via traditional phlebotomy.

The second document lists all tests currently planned to potentially be run on samples collected in Theranos Wellness Centers during Phase I of Theranos' operations.

Please note these documents only pertain to samples collected through Theranos Wellness Centers (Theranos' Patient Service Center retail locations).

As always, please let me know if there are any clarifications we can provide on the above or the attached prior to circulation.

Please also note that these documents are Theranos Confidential and Proprietary and we ask for your protection of them as with the other materials we have shared, including protection from public disclosure consistent with FDA regulations and Exemption 4 to the Freedom of Information Act.

With my best regards,
Elizabeth

THERANOS CONFIDENTIAL

Tests run in Theranos' CLIA laboratory from Samples collected in Theranos Wellness Centers

Test	Chemistry	Device
CBC	Theranos	FDA cleared/approved
CMP	Theranos Protocol*	FDA cleared/approved
Lipid	Theranos Protocol	FDA cleared/approved
Urinalysis	FDA cleared/approved	FDA cleared/approved
TSH	Theranos	TSPU
PSA	Theranos	TSPU
VitD	Theranos	TSPU
HbA1c	FDA cleared/approved	FDA cleared/approved
Uric Acid	FDA cleared/approved	FDA cleared/approved
DHEA-S	FDA cleared/approved	FDA cleared/approved
Total, Testosterone	FDA cleared/approved	FDA cleared/approved
Free, Testosterone	FDA cleared/approved	FDA cleared/approved

* Theranos Protocol means FDA-cleared assays running a Theranos protcol (i.e. modified under the CLIA regulations). These assays are thus validated as LDTs.

THERANOS CONFIDENTIAL

Tests planned to be run in Theranos' CLIA laboratory from Samples collected in Theranos Wellness Centers

CPT	Test Name	Chemistry	Device	Theranos Internal Methodology Name/Test Category	Matrix
85014	hematocrit	Theranos	TSPU	Cytometry	whole blood
85045	automated reticulocyte count	Theranos	TSPU	Cytometry	whole blood
85049	automated platelet count	Theranos	TSPU	Cytometry	whole blood
85049	mean platelet volume	Theranos	TSPU	Cytometry	whole blood
85048	automated leukocyte count	Theranos	TSPU	Cytometry	whole blood
85041	automated rbc count	Theranos	TSPU	Cytometry	whole blood
90006	mean cell volume	Theranos	TSPU	Cytometry	whole blood
90007	mean corpuscular hemoglobin	Theranos	TSPU	Cytometry	whole blood
90008	mean corpuscular hemoglobin per cell	Theranos	TSPU	Cytometry	whole blood
90011	red cell distribution width	Theranos	TSPU	Cytometry	whole blood
90003	basophils	Theranos	TSPU	Cytometry	whole blood
90004	eosinophils	Theranos	TSPU	Cytometry	whole blood
90005	lymphocytes	Theranos	TSPU	Cytometry	whole blood
90009	monocytes	Theranos	TSPU	Cytometry	whole blood
90010	neutrophils	Theranos	TSPU	Cytometry	whole blood
81001-9	RBC, urine	Theranos	TSPU	Cytometry	urine
81001-10	WBC, urine	Theranos	TSPU	Cytometry	urine
81001-11	Epithelial Cells, urine (squamous, non-squamous, transitional, renal)	Theranos	TSPU	Cytometry	urine
81001-12	Bacteria, urine	Theranos	TSPU	Cytometry	urine
81001-13	Casts, urine (10 types)	Theranos	TSPU	Cytometry	urine
81001-14	Crystals, urine (10 types)	Theranos	TSPU	Cytometry	urine
81001-15	Budding yeast, urine	Theranos	TSPU	Cytometry	urine
81001-16	Hypae yeast, urine	Theranos	TSPU	Cytometry	urine
81001-17	Trichomonas, urine	Theranos	TSPU	Cytometry	urine
81001-18	Oval fat bodies, urine	Theranos	TSPU	Cytometry	urine
81001-19	Fat bodies, urine	Theranos	TSPU	Cytometry	urine
81001-20	Mucous, urine	Theranos	TSPU	Cytometry	urine
81001-20	Sperm, urine	Theranos	TSPU	Cytometry	urine
86360	t cell, absolute count/ratio	Theranos	TSPU	Cytometry	whole blood
86359	t cells, total count	Theranos	TSPU	Cytometry	whole blood
85384	fibrinogen	FDA cleared/approved	FDA cleared/approved	Cytometry	whole blood
86355	b cells, total count	Theranos	TSPU	Cytometry	whole blood
86357	nk cells, total count	Theranos	TSPU	Cytometry	whole blood
87177	Ova and Parasites Smears	Manual	Manual	Cytometry	multiple
85007	Manual Differential	Manual	Manual	Cytometry	whole blood
85018	hemoglobin	Theranos	TSPU	General Chemistry	whole blood
82947	glucose, blood quant	Theranos Protocol*	FDA cleared/approved	General Chemistry	whole blood
82565	creatinine (egfr)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82565	creatinine	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84520	urea nitrogen	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84132	potassium, serum	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84295	sodium, serum	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82435	chloride, blood	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82374	carbon dioxide, blood	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
NA	anion gap	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84460	alanine amino (alt) (sgpt)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82310	calcium	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84450	transferase (ast) (sgot)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82040	albumin, serum	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
NA	globulin	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84075	alkaline phosphatase	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82247	bilirubin, total	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84155	protein, serum	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82465	cholesterol, bld/serum	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
83718	lipoprotein (HDL)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84478	triglycerides	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82042	albumin, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
84156	protein, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
82340	calcium in urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
82436	chloride, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
83069	hemoglobin, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
84578	urobilinogen, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
84583	urobilinogen, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-1	nitrite	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-2	pH	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-3	Specific Gravity	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-4	Ketones (Acetoacetate)	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-5	Ketones (beta-hydroxybutyrate)	Theranos	TSPU	General Chemistry	urine
81001-6	Leukocyte Esterase	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-7	Bilirubin	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
81001-8	Glucose	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine

82248	bilirubin, direct	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood, urine
85610	prothrombin time	Theranos	TSPU	General Chemistry	whole blood
85652	rbc sed rate, automated	Theranos	TSPU	General Chemistry	whole blood
82550	ck (cpk)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82570	creatinine, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
NA	Microalbumin/Creatinine ratio	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
83540	iron	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
83735	magnesium	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84100	phosphorus	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84550	uric acid, blood	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
83721	lipoprotein, blood (LDL)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
85730	thromboplastin time, partial	Theranos	TSPU	General Chemistry	whole blood
83550	iron binding	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
86901	blood typing, rh (d)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
86900	blood typing, abo	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
83655	Assay of lead	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
86850	rbc antibody screen	Theranos	TSPU	General Chemistry	whole blood
83615	lactate dehydrogenase (ld) (ldh) enzyme	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82150	amylase	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
83690	lipase	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82055	ethanol	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82977	ggt	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
86403-1	streptozyme	Theranos	TSPU	General Chemistry	whole blood
82003	acetaminophen	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
80196	salicylate	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
80178	lithium	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
86880	coombs (antiglobulin test, agt)	Theranos	TSPU	General Chemistry	whole blood
84580	urine urobilinogen	Theranos	TSPU	General Chemistry	urine
84300	sodium, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
82140	ammonia	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84560	uric acid, urine	Theranos	TSPU	General Chemistry	urine
84630	zinc	Theranos	TSPU	General Chemistry	whole blood, urine
82085	aldolase	Theranos	TSPU	General Chemistry	whole blood
82507	citrate	Theranos	TSPU	General Chemistry	whole blood
85300	antithrombin iii activity; clotting inhibitors or anticoagulants	Theranos	TSPU	General Chemistry	whole blood
82145	amphetamines	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
83945	oxalate	Theranos	TSPU	General Chemistry	whole blood
83605	lactic acid	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
84133	potassium, urine	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
84540	urea-nitrogrn, urine	Theranos	TSPU	General Chemistry	urine
82955	g6pd enzyme	Theranos	TSPU	General Chemistry	whole blood
82803-1	blood gases: ph	Theranos	TSPU	General Chemistry	whole blood
82803-3	blood gases: pco2	Theranos	TSPU	General Chemistry	whole blood
82803-4	base excess / deficit	Theranos	TSPU	General Chemistry	whole blood
82009	acetone/ketones	Theranos	TSPU	General Chemistry	whole blood
84210	pyruvate	Theranos	TSPU	General Chemistry	whole blood
83719	blood lipoprotein (VLDL)	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82180	ascorbic acid	Theranos	TSPU	General Chemistry	whole blood
82239	bile acids, total	Theranos	TSPU	General Chemistry	whole blood
85520	heparin assay	Theranos	TSPU	General Chemistry	whole blood
82725	fatty acids, blood	Theranos	TSPU	General Chemistry	whole blood
82965	gdh enzyme	Theranos	TSPU	General Chemistry	whole blood
84430	thiocyanate	Theranos	TSPU	General Chemistry	urine
84060	Assay acid phosphatase	Theranos Protocol	FDA cleared/approved	General Chemistry	whole blood
82043	Microalbumin	FDA cleared/approved	FDA cleared/approved	General Chemistry	urine
84443	thyroid stim hormone	Theranos	TSPU	ELISA	whole blood
83036	glycosylated hemoglobin	Theranos	TSPU	ELISA	whole blood
82306	vitamin d, 25 hydroxy	Theranos	TSPU	ELISA	whole blood
84153	psa, total	Theranos	TSPU	ELISA	whole blood
84439	thyroxine, free	Theranos	TSPU	ELISA	whole blood
84702	chorionic gonadotropin	Theranos	TSPU	ELISA	whole blood
86140	c-reactive protein	Theranos	TSPU	ELISA	whole blood
82728	ferritin	Theranos	TSPU	ELISA	whole blood
82607	vitamin b-12	Theranos	TSPU	ELISA	whole blood
87340	hepatitis b surface ag, eia	Theranos	TSPU	ELISA	whole blood
81025	pregnancy , urine	Theranos	TSPU	ELISA	urine
86592	syphilis non-trep qual	Theranos	TSPU	ELISA	whole blood
86703	hiv-1/hiv-2, single	Theranos	TSPU	ELISA	whole blood
86618	lyme disease antibody (IgG and IgM)	Theranos	TSPU	ELISA	whole blood
84484	troponin, quant (tCNI)	Theranos	TSPU	ELISA	whole blood
84484	troponin, quant (tCNT)	Theranos	TSPU	ELISA	whole blood
84436	thyroxine, total	Theranos	TSPU	ELISA	whole blood
86803	hepatitis c ab	Theranos	TSPU	ELISA	whole blood
82746	folic acid serum, blood	Theranos	TSPU	ELISA	whole blood
86038	antinuclear antibodies (IgG)	Theranos	TSPU	ELISA	whole blood

83001	gonadotropin (fsh)	Theranos	TSPU	ELISA	whole blood
84403	total testosterone	Theranos	TSPU	ELISA	whole blood
86762	rubella antibody, IgM	Theranos	TSPU	ELISA	whole blood
86317	rubella antibody IgG	Theranos	TSPU	ELISA	whole blood
82670	estradiol	Theranos	TSPU	ELISA	whole blood
84479	thyroid (t3 or t4)	Theranos	TSPU	ELISA	whole blood
82553	creatine, mb fraction	Theranos	TSPU	ELISA	whole blood
84146	prolactin	Theranos	TSPU	ELISA	whole blood
84480	triiodothyronine (t3)	Theranos	TSPU	ELISA	whole blood
83002	gonadotropin (lh)	Theranos	TSPU	ELISA	whole blood
80101-5	dolophine (methadone)	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-16	methadone metabolite	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-7	heroin (opiate screen)	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-8	Cocaine	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-2	Ethyl glucuronide	Theranos	TSPU	ELISA	urine
80101-10	Amphetamines	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-11	Phencyclidine (PCP)	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-12	Marijuana (THC)	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-14	Ecstasy	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
80101-15	Tricyclic Antidepressants	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
86235-01	nuclear antigen antibody, SSB	Theranos	TSPU	ELISA	whole blood
86235-02	nuclear antigen antibody, SSA	Theranos	TSPU	ELISA	whole blood
86235-03	nuclear antigen antibody, Sm	Theranos	TSPU	ELISA	whole blood
86235-04	nuclear antigen antibody, RNP	Theranos	TSPU	ELISA	whole blood
86235-05	nuclear antigen antibody, Scl-70	Theranos	TSPU	ELISA	whole blood
86235-06	nuclear antigen antibody, Jo-1	Theranos	TSPU	ELISA	whole blood
86706	hep b surface antibody	Theranos	TSPU	ELISA	whole blood
83970	parathormone	Theranos	TSPU	ELISA	whole blood
86308	heterophile antibodies (IgM)	Theranos	TSPU	ELISA	whole blood
87430	strep a ag, eia	Theranos	TSPU	ELISA	nasal
82105	alpha-fetoprotein, serum	Theranos	TSPU	ELISA	whole blood
87400-1	influenza a/b, ag, eia	Theranos	TSPU	ELISA	whole blood
87400-2	influenza a/b, ag, eia	Theranos	TSPU	ELISA	whole blood
86431	rheumatoid factor, quant (IgM)	Theranos	TSPU	ELISA	whole blood
84144	progesterone	Theranos	TSPU	ELISA	whole blood
86787	varicella-zoster antibody	Theranos	TSPU	ELISA	whole blood
84466	transferrin	Theranos	TSPU	ELISA	whole blood
84402	testosterone	Theranos	TSPU	ELISA	whole blood
86665-1	epstein-barr antibody IgG	Theranos	TSPU	ELISA	whole blood
86665-2	epstein-barr antibody IgM	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86704	hep b core antibody, total	Theranos	TSPU	ELISA	whole blood
85379	fibrin degradation, quant	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86376	microsomal antibody	Theranos	TSPU	ELISA	whole blood
84481	ft-3, free	Theranos	TSPU	ELISA	whole blood
86709	hep a antibody, igm	Theranos	TSPU	ELISA	whole blood
86800	thyroglobulin antibody, IgG	Theranos	TSPU	ELISA	whole blood
86705	hep b core antibody, igm	Theranos	TSPU	ELISA	whole blood
82785	ige	Theranos	TSPU	ELISA	whole blood
86430-1	rheumatoid factor test, IgG	Theranos	TSPU	ELISA	whole blood
86430-2	rheumatoid factor test, IgA	Theranos	TSPU	ELISA	whole blood
86747-01	parvovirus antibody, IgG	Theranos	TSPU	ELISA	whole blood
82784-1	iga	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
82784-2	igg	Theranos	TSPU	ELISA	whole blood
82784-3	igm	Theranos	TSPU	ELISA	whole blood
82784-4	igd	Theranos	TSPU	ELISA	whole blood
82784-5	igd	Theranos	TSPU	ELISA	whole blood
86708	hep a antibody, total	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86696	herpes simplex type 2	Theranos	TSPU	ELISA	whole blood
86695	herpes simplex	Theranos	TSPU	ELISA	whole blood
86147	cardiolipin antibody	Theranos	TSPU	ELISA	whole blood
82378	carcinoembryonic antigen	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
82677	estriol	Theranos	TSPU	ELISA	whole blood
82533	total cortisol	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
83880	natriuretic peptide	Theranos	TSPU	ELISA	whole blood
86039	antinuclear antibodies (ana)	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
83925	opiates	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
82627	dehydroepiandrosterone sulfate (dheas)	Theranos	TSPU	ELISA	whole blood
86765-01	rubeola antibody, IgG	Theranos	TSPU	ELISA	whole blood
86765-02	rubeola antibody, IgM	Theranos	TSPU	ELISA	whole blood
83525	insulin	Theranos	TSPU	ELISA	whole blood
86735	mumps antibody, IgG	Theranos	TSPU	ELISA	whole blood
86677-2	h pylori iab igg	Theranos	TSPU	ELISA	whole blood
86225	dna antibody	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86664	epstein-barr antibody, nuclear antigen, IgG	Theranos	TSPU	ELISA	whole blood
84270	sex hormone globul	Theranos	TSPU	ELISA	whole blood

83090	homocysteine	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
80164	dipropylacetic acid	Theranos	TSPU	ELISA	whole blood, urine
80154	benzodiazepines	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
84432	thyroglobulin	Theranos	TSPU	ELISA	whole blood
86663	epstein-barr antibody, early antigen, IgG	Theranos	TSPU	ELISA	whole blood
84154	psa, free	FDA cleared/approved	FDA cleared/approved	ELISA	urine
86644	cmv antibody, IgG	Theranos	TSPU	ELISA	whole blood
84134	prealbumin	Theranos	TSPU	ELISA	whole blood
80185	phenytoin, total	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
86645	cmv antibody, igm	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86780	Treponema pallidum	Theranos	TSPU	ELISA	whole blood
82232	beta-2 protein	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
82103	alpha-1-antitrypsin, total	Theranos	TSPU	ELISA	whole blood
80156	carbamazepine, total	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
83010	haptoglobin, quant	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
86060	antistreptolysin o, titer	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
80162	digoxin	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
83874	myoglobin	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
83840	methadone	Theranos	TSPU	ELISA	whole blood, urine
80202	vancomycin	Theranos	TSPU	ELISA	whole blood, urine
86160-3	complement component 3 antigen	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
86160-4	complement component 4 antigen	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
82205	barbiturates	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
84681	c-peptide	Theranos	TSPU	ELISA	whole blood
83003	growth hormone (hgh)	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
82668	erythropoietin	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
80184	phenobarbital	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
85302	protein c, antigen; clotting inhibitors or anticoagulants	Theranos	TSPU	ELISA	whole blood
84080	Assay alkaline phosphatases	Theranos	TSPU	ELISA	whole blood
83992	phencyclidine	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood, urine
83887	Assay of nicotine -Urine	Theranos	TSPU	ELISA	urine
83887	Assay of nicotine - serum	Theranos	TSPU	ELISA	whole blood
82172-1	apo a-1	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
82172-2	apo b	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
82941	gastrin	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
80200	tobramycin	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
80198	theophylline	Theranos Protocol	FDA cleared/approved	ELISA	whole blood, urine
82308	calcitonin	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
84442	Assay of thyroid activity	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
85385	Fibrinogen	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
87274	herpes simplex 1, ag, if	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
80170	gentamicin	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
86790-2	dengue fever virus antibodies, IgM	Theranos	TSPU	ELISA	whole blood
86790-3	dengue fever virus antibodies, IgG	Theranos	TSPU	ELISA	whole blood
82610	cystatin c	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
86788	west nile virus ab, igm	Theranos	TSPU	ELISA	whole blood
87273	herpes simplex 2, ag, if	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86619	Borrelia antibody	FDA cleared/approved	FDA cleared/approved	ELISA	whole blood
86753-1	Trypanosoma cruzi IgG_Chagas	Theranos	TSPU	ELISA	whole blood
83520-1	TNF alpha	Theranos	TSPU	ELISA	whole blood
83520-2	IL-6	Theranos	TSPU	ELISA	whole blood
83520-3	IL-8	Theranos	TSPU	ELISA	whole blood
83520-4	IL-10	Theranos	TSPU	ELISA	whole blood
83520-5	IL-12	Theranos	TSPU	ELISA	whole blood
83520-6	IFN gamma	Theranos	TSPU	ELISA	whole blood
84080-2	BSAP	Theranos	TSPU	ELISA	whole blood
83520-7	M30	Theranos	TSPU	ELISA	whole blood
83520-8	M65	Theranos	TSPU	ELISA	whole blood
83520-9	ENA-78 (CXCL5)	Theranos	TSPU	ELISA	whole blood
83520-10	Eotaxin (CCL11)	Theranos	TSPU	ELISA	whole blood
83520-11	RANTES (CCL5)	Theranos	TSPU	ELISA	whole blood
83520-12	TARC (CCL17)	Theranos	TSPU	ELISA	whole blood
83520-13	GLP-1	Theranos	TSPU	ELISA	whole blood
83520-14	IL-1 beta	Theranos	TSPU	ELISA	whole blood
83520-15	Leptin	Theranos	TSPU	ELISA	whole blood
83520-16	VEGF-R2	Theranos	TSPU	ELISA	whole blood
52005	IGF-1	Theranos	TSPU	ELISA	whole blood
G0431QW	Cotinine, Serum and Urine	Theranos	TSPU	ELISA	whole blood, urine
82985	Alpha-1-Acid Glycoprotein	Theranos Protocol	FDA cleared/approved	ELISA	whole blood
87491	chylmd trach, dna, amp probe	FDA cleared/approved	FDA cleared/approved	TNAA	urine
87591	n.gonorrhoeae, dna, amp prob	FDA cleared/approved	FDA cleared/approved	TNAA	urine
87536	hiv-1, dna, quant	FDA cleared/approved	FDA cleared/approved	TNAA	whole blood
87522	hepatitis c, rna, quant	FDA cleared/approved	FDA cleared/approved	TNAA	whole blood
87517	hepatitis b, dna, quant	FDA cleared/approved	FDA cleared/approved	TNAA	whole blood
87798	Blood Culture Identification (BCID) Panel	FDA cleared/approved	FDA cleared/approved	TNAA	whole blood

H5N1 flu	Theranos	TSPU	TNAA	respiratory
H7N9 flu - HA gene	Theranos	TSPU	TNAA	respiratory
H7N9 flu - NA gene	Theranos	TSPU	TNAA	respiratory
Influenza A - MP	Theranos	TSPU	TNAA	respiratory
Influenza B - MP	Theranos	TSPU	TNAA	respiratory
Influenza H1N1 novel (2009) - HA	Theranos	TSPU	TNAA	respiratory
Influenza H1N1 seasonal - HA	Theranos	TSPU	TNAA	respiratory
Influenza H3N2 - HA	Theranos	TSPU	TNAA	respiratory
Bocavirus 1 + 3	Theranos	TSPU	TNAA	respiratory
Bocavirus 2 + 4	Theranos	TSPU	TNAA	respiratory
Coronavirus MERS	Theranos	TSPU	TNAA	respiratory
Metapneumovirus A	Theranos	TSPU	TNAA	respiratory
Metapneumovirus B	Theranos	TSPU	TNAA	respiratory
Parainfluenza 1	Theranos	TSPU	TNAA	respiratory
Parainfluenza 2	Theranos	TSPU	TNAA	respiratory
Parainfluenza 3	Theranos	TSPU	TNAA	respiratory
Parainfluenza 4a	Theranos	TSPU	TNAA	respiratory
Parainfluenza 4b	Theranos	TSPU	TNAA	respiratory
RSV A	Theranos	TSPU	TNAA	respiratory
RSV B	Theranos	TSPU	TNAA	respiratory
Adenovirus B	Theranos	TSPU	TNAA	respiratory
Adenovirus C	Theranos	TSPU	TNAA	respiratory
Adenovirus E	Theranos	TSPU	TNAA	respiratory
Coronavirus 229E	Theranos	TSPU	TNAA	respiratory
Coronavirus HKU1	Theranos	TSPU	TNAA	respiratory
Coronavirus NL63	Theranos	TSPU	TNAA	respiratory
Coronavirus OC43	Theranos	TSPU	TNAA	respiratory
Measles	Theranos	TSPU	TNAA	respiratory, urine
Mumps	Theranos	TSPU	TNAA	respiratory, urine
Rhinovirus A	Theranos	TSPU	TNAA	respiratory
Rhinovirus B	Theranos	TSPU	TNAA	respiratory
Rhinovirus C	Theranos	TSPU	TNAA	respiratory
Rubella	Theranos	TSPU	TNAA	respiratory, blood
Streptococcus pyogenes (strep A)	Theranos	TSPU	TNAA	respiratory
Mycobacterium abscessus	Theranos	TSPU	TNAA	respiratory, blood
Bordetella parapertussis	Theranos	TSPU	TNAA	respiratory
Bordetella pertussis	Theranos	TSPU	TNAA	respiratory
Bordetella holmesii	Theranos	TSPU	TNAA	respiratory
Chlamydophila pneumoniae	Theranos	TSPU	TNAA	respiratory
Enterobacter aerogenes	Theranos	TSPU	TNAA	respiratory
Enterobacter cloacae	Theranos	TSPU	TNAA	respiratory
H. influenzae blaROB	Theranos	TSPU	TNAA	respiratory, blood
H. influenzae blaTEM	Theranos	TSPU	TNAA	respiratory, blood
Haemophilus influenzae	Theranos	TSPU	TNAA	respiratory, blood
Haemophilus parainfluenzae	Theranos	TSPU	TNAA	respiratory, blood
Legionella pneumophila	Theranos	TSPU	TNAA	respiratory
Mycoplasma pneumoniae	Theranos	TSPU	TNAA	respiratory
Penicillin-resistant S. pneumo	Theranos	TSPU	TNAA	respiratory
Streptococcus pneumoniae	Theranos	TSPU	TNAA	respiratory
Acinetobacter baumannii	Theranos	TSPU	TNAA	respiratory, blood
Burkholderia cepacia	Theranos	TSPU	TNAA	respiratory
Klebsiella pneumoniae	Theranos	TSPU	TNAA	respiratory, blood, urine
KPC resistance gene	Theranos	TSPU	TNAA	respiratory, blood, urine
Moraxella catarrhalis	Theranos	TSPU	TNAA	respiratory
MRSA	Theranos	TSPU	TNAA	respiratory
MTB	Theranos	TSPU	TNAA	respiratory
Serratia marcescens	Theranos	TSPU	TNAA	respiratory, blood, urine
Staphylococcus aureus	Theranos	TSPU	TNAA	respiratory
Staphylococcus aureus (Vancomycin-resistant) VRSA	Theranos	TSPU	TNAA	respiratory
Clostridium sordellii	Theranos	TSPU	TNAA	respiratory, blood
Pseudomonas aeruginosa	Theranos	TSPU	TNAA	respiratory, blood, urine
Vancomycin-resistant Enterococci (VRE)	Theranos	TSPU	TNAA	respiratory, blood, urine
Dengue Virus 1	Theranos	TSPU	TNAA	blood
Dengue Virus 2	Theranos	TSPU	TNAA	blood
Dengue Virus 3	Theranos	TSPU	TNAA	blood
Dengue Virus 4	Theranos	TSPU	TNAA	blood
Pan Plasmodium	Theranos	TSPU	TNAA	blood
Trypanosoma cruzi	Theranos	TSPU	TNAA	blood
West Nile Virus 1	Theranos	TSPU	TNAA	blood
West Nile Virus 2	Theranos	TSPU	TNAA	blood
HIV-1 Group M (Assay 1 & 2)	Theranos	TSPU	TNAA	blood
HIV-2 Group A	Theranos	TSPU	TNAA	blood
HIV-2 Group B	Theranos	TSPU	TNAA	blood
Hepatitis A	Theranos	TSPU	TNAA	blood
HepDelta	Theranos	TSPU	TNAA	blood

Epstein-Barr Virus	Theranos	TSPU	TNAA	blood
HSV1/HSV2	Theranos	TSPU	TNAA	blood
Streptococcus agalactae (strep B)	Theranos	TSPU	TNAA	urine
Trichomonas vaginalis	Theranos	TSPU	TNAA	urine
Varicella-zoster	Theranos	TSPU	TNAA	blood

* Theranos Protocol means FDA-cleared assays running a Theranos protcol (i.e. modified under the CLIA regulations). These assays are thus validated as LDTs.

EXHIBIT 9

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
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U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

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Title of Invention	Devices, Methods and Systems for Reducing Sample Volume
Attorney Docket Number (if applicable)	3013.101

Correspondence Address

Direct all correspondence to (select one):

(●) The address corresponding to Customer Number	( ) Firm or Individual Name
Customer Number	107075

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- (●) No.
- ( ) Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:
- ( ) Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

Doc Code: **TR.PROV**
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Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

○ Applicant asserts small entity status under 37 CFR 1.27

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Signature	/James Fox/			Date (YYYY-MM-DD)	2013-10-08
First Name	James	Last Name	Fox	Registration Number (If appropriate)	38455

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ABSTRACT

DEVICES, METHODS AND SYSTEMS FOR REDUCING SAMPLE VOLUME

Devices, methods and systems are provided for reducing the sample volume required for analysis. Inserts placed within a sample container, and substitute sample containers having smaller volume sample chambers are provided. Methods are provided for detection and quantification of target substances in reduced volume samples. Methods include reducing the volume of sample, and: increasing illumination; increasing dye concentration or amount; increasing the amount of an enzyme substrate; increasing the amounts, concentration, or labeling of antibodies for detection; increasing optical detector sensitivity; increasing the path length of light passing through the sample; decreasing the separation between the sample and an optical detector; altering the wavelength, or polarization, or number of wavelengths, passing through the sample; increasing electronic amplification of electrical signals; altering assay temperature; and other alterations.

Attorney Docket: 3013.101

DEVICES, METHODS AND SYSTEMS FOR REDUCING SAMPLE VOLUME

BACKGROUND

[0001] Detection of analytes in a sample and determination of the chemical composition of a sample are useful in many clinical and scientific applications. Thus, methods, devices, and systems for analyzing samples and for detecting target substances within the samples are useful in many contexts. A method of testing for, or for detecting, a target substance in a sample may be termed an "assay." Some assays may be performed by devices or systems with little or no human intervention; such assays may be termed automated assays, and are performed by automatic devices or automatic systems.

[0002] Clinical assays are often developed to identify target materials in samples taken from patients. For example, targets may include proteins, nucleic acids, lipids, organic molecules, inorganic molecules and ions. Such target materials may include drugs, drug metabolites, vitamins, hormones, growth factors, carrier proteins, cells, infectious agents, and other target materials that may be indicative of medical conditions or disorders. Other clinical assays may be directed to testing for levels of drugs, drug metabolites, hormones, vitamins, or other substances which may be of therapeutic or clinical interest. In some instances, a sample may include multiple analytes, and multiple assays may be required to detect or quantify all of the analytes of interest in a sample.

[0003] Clinical assays require samples to be obtained from a subject, such as a patient suffering from, or suspected of suffering from, a disease characterized by markers identifiable by the assay. However, providing samples is often uncomfortable, or difficult, or inconvenient for a subject; the discomfort, difficulty, and inconvenience is typically greater the larger the volume of sample that is required.

[0004] Accordingly, methods and devices for reducing the volume of sample required to be obtained from a subject are desired, and assays, guidelines and methods for altering existing

machines and assays that may be performed on smaller volume samples than presently practiced are also desired.

INCORPORATION BY REFERENCE

[0005] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY

[0006] Applicants provide methods and devices for modifying assays, and for modifying assay devices, to reduce the volume of sample required for the performance of assays for the detection of analytes. Devices and systems modified according to the methods disclosed herein, and using the devices disclosed herein, are able to perform analytical assays while requiring less sample, providing greater comfort to subjects since smaller samples are less painful to obtain; providing more cost-effective assays and analyses, since smaller sample volumes typically require less reagent volume, and so are less costly to assay; providing less waste pursuant to the assays; and providing other advantages as compared to original assays and methods which require greater sample volumes.

[0007] Applicants provide methods for modifying a clinical analysis device comprising reducing the sample volume by including an insert into a sample container. Applicants provide methods for modifying a sample container used in a clinical analysis device comprising reducing the sample volume by including an insert into the sample container. Applicants provide methods for modifying sample analysis in a clinical analysis device comprising reducing the sample volume analyzed by the clinical analysis device by including an insert into the sample container. Applicants provide methods for modifying sample analysis in a clinical analysis device comprising reducing the sample volume analyzed by the clinical analysis device by providing a substitute sample container having a smaller volume than the original sample container.

[0008] Methods for reducing the volume of sample used during, or required for, sample analysis include providing an insert which fits in a sample container effective to reduce the volume of sample held by the combined sample container and insert; or providing a substitute sample container which has a smaller volume than the original sample container. The sample

may be contained within a sample container during at least a portion of the performance of said original assay, said sample container comprising an internal cavity for holding said sample, said internal cavity having a volume, and wherein any of the foregoing methods comprise reducing said volume of said internal cavity. In embodiments, reducing the volume of said internal cavity comprises providing an alternative sample container. In embodiments, reducing the volume of said internal cavity comprises placing an insert into said internal cavity. In embodiments, an insert may comprise an insert cavity configured to hold said sample, wherein said insert cavity comprises a volume less than said internal cavity volume. In embodiments, an insert may be configured effective that optical signals indicative of the presence of, or quantification of, a target substance in a sample contained within said insert may be detected by said optical detector.

[0009] Applicants further provide devices for reducing the volume of sample held within a sample container, wherein said sample container is configured to hold a first volume of sample effective to allow detection of a target substance in said sample by a detector disposed externally to said sample container, wherein said device comprises an insert configured to i) fit within said sample container, ii) hold a second volume of sample, wherein said second volume of sample is less than said first volume of sample, and iii) allow the detection of a signal indicative of the presence of, or quantification of, a target substance in a sample contained within said insert, wherein said detection comprises detection by a detector disposed externally to said sample container. In embodiments, the insert is configured to allow the passage of light effective that optical signals indicative of the presence of, or quantification of, a target substance in a sample contained within said insert may be detected by an optical detector disposed externally to the sample container.

[0010] Applicants provide methods for modifying a clinical analysis device comprising reducing the sample volume by including an insert into a sample holder, and increasing the sensitivity of a detector. Applicants provide methods for modifying a clinical analysis device comprising reducing the sample volume by including an insert into a sample holder, and decreasing the distance between a sample and a detector. Applicants provide methods for modifying a clinical analysis device comprising reducing the sample volume by including an insert into a sample holder, and increasing the intensity of an illumination source providing illumination of a sample to be detected by a detector. Applicants provide methods for modifying

a clinical analysis device comprising reducing the volume of a sample container by placing an insert into said sample container; and increasing the concentration of a dye or of a substrate which is detected by, or which provides a signal detected by, a detector during operation of the device. Applicants provide methods for modifying a clinical analysis device comprising diluting a sample, and: increasing the sensitivity of a detector; or increasing the intensity of an illumination source providing illumination of a sample to be detected by a detector; or increasing the concentration of a dye/substrate which is detected by a detector during operation of the device.

[0011] Accordingly, Applicants provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the intensity of illumination applied to said sample, as compared to the original intensity of illumination applied to the sample, wherein said illumination is used to detect the presence of said target substance in the sample, or to quantify the amount of said target substance in the sample.

[0012] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a fluorescent label for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the intensity of light illuminating the sample and assay reagents during fluorescence measurements, as compared to the original intensity of light illuminating the sample and assay reagents during fluorescence measurements, wherein said fluorescence measurements are used to detect the presence of said target substance in the sample, or to quantify the amount of said target substance in the sample.

[0013] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a dye for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the concentration of dye added to the sample, as compared to the original concentration of dye added to the sample, wherein said dye labels a target substance in the sample. In embodiments, increasing the concentration of a dye added to the sample comprises increasing the amount of dye added to the sample, as compared to the original amount of dye added to the sample.

[0014] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an enzymatic label for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the concentration of substrate added to the sample in the presence of the enzyme prior to, or during, enzymatic label measurements, as compared to the original concentration of substrate added to the sample, wherein said enzymatic label measurements are indicative of the presence of, or quantities of, target substance in the sample.

[0015] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a labeled antibody for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the concentration of labeled antibody added to the sample, as compared to the original concentration of labeled antibody added to the sample, wherein said antibody binds a target substance in the sample. In embodiments, increasing the concentration of a labeled antibody added to the sample comprises increasing the amount of labeled antibody added to the sample, as compared to the original amount of labeled antibody added to the sample.

[0016] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a labeled antibody for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the number of labels per labeled antibody added to the sample, as compared to the original number of labels per labeled antibody added to the sample, wherein said antibody binds a target substance in the sample.

[0017] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target

substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the sensitivity of an optical detector used to detect said optical signal, as compared to the original sensitivity of the optical detector used to detect the optical signal.

[0018] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Decreasing the separation between the sample and an optical detector used to detect said optical signal, as compared to the original separation between the sample and the optical detector used to detect the optical signal.

[0019] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the path length within the sample between a source of illumination and through the sample to an optical detector used to detect said optical signal, as compared to the original path length within the sample between a source of illumination and through the sample to an optical detector used to detect the optical signal. In embodiments, the path length within the sample is increased, as compared to the original path length within the sample, by alteration of the container holding the sample. In further embodiments, the path length within the sample is increased, as compared to the original path length within the sample, by reflection or refraction of light within the container holding the sample.

[0020] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Altering the wavelength of light passing through said sample and to an optical detector used to detect the optical signal, as compared to the original wavelength of light passing through the sample and to an optical detector used to detect the optical signal. In embodiments, altering the wavelength of light passing through said sample and to an optical detector used to detect the optical signal comprises providing multiple wavelengths of light passing through the sample and to an optical detector.

[0021] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Altering the polarization of light passing through said sample and to an optical detector used to detect the optical signal, as compared to the original polarization of light passing through the sample and to an optical detector used to detect the optical signal.Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an electrical signal indicative of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the electronic amplification of said electrical signal, as compared to the original electronic amplification of the electrical signal. In embodiments, the electrical signal is produced by the target substance, or by a reagent which binds to or reacts with said target substance.

[0022] Applicants further provide a method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a signal indicative of the presence of, or quantification of, the target substance in a sample, wherein said signal comprise a temperature-sensitive signal, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Altering the temperature of the assay. In embodiments, the temperature-sensitive signal is increased with increasing temperature, and wherein altering the temperature of the assay comprises increasing the temperature of the assay. In further embodiments, the temperature of said original performance of said assay was near 20 °C, and wherein said altered assay temperature is selected from about 25 °C, about 30 °C, about 32 °C, about 34 °C, about 35 °C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, and about 40 °C.

[0023] In any of the foregoing methods, the sample may be contained within a sample container during at least a portion of the performance of said original assay, said sample container comprising an internal cavity for holding said sample, said internal cavity having a volume, and wherein any of the foregoing methods comprise reducing said volume of said internal cavity. In embodiments, reducing the volume of said internal cavity comprises providing an alternative sample container. In embodiments, reducing the volume of said internal cavity comprises placing an insert into said internal cavity. In embodiments, an insert may comprise an insert cavity configured to hold said sample, wherein said insert cavity comprises a volume less than said internal cavity volume. In embodiments, an insert may be configured effective that optical signals indicative of the presence of, or quantification of, a target substance in a sample contained within said insert may be detected by said optical detector.

[0024] Practice of these methods provides surprising results. For example, devices designed to use large volume samples (e.g., volumes on the order of tens of milliliters, or even on the order of one or a few milliliters) may be modified to provide results using only small fractions of a milliliter, or even only a few microliters of sample. Practice of the methods disclosed herein, and use of the devices disclosed herein, allows detection of target analytes in very small samples; this is surprising since previously available devices, systems, assays, and techniques required much larger sample volumes. In addition, use of small volume samples may allow detection of target analytes within short periods of time, including within periods of time shorter than the times required using unmodified devices and systems.

[0025] Practice of these methods provides advantages over previous methods and devices by requiring smaller volumes of reagents as well as smaller volumes of sample. Thus, the methods and devices disclosed herein provide synergistic advantages, in that by reducing the sample volume required, reagent volumes are also reduced, assay cost is reduced, discomfort to the subject (from whom the sample is obtained) is reduced, and waste resulting from the performance of the assays is reduced. Accordingly, the present methods and devices are useful and provide surprising advantages for assays for detecting analytes in a sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Fig. 1 provides an illustration of an insert configured to fit within a sample container.

[0027] Fig. 2A shows a perspective view of an insert having features as disclosed herein.

[0028] Fig. 2B shows a cross-section of an insert as shown in Fig. 2A, showing the insert cavity and illustrating an example of a bevel in the floor of an insert suitable for enhancing fluid flow and for reducing the volume of an insert cavity.

[0029] Fig. 3A provides an illustration of a system including an analysis device, a sample container, and an insert configured to fit within the sample container. The sample is held in the insert cavity of the insert, and so a smaller amount of sample is used than would be used in the absence of the insert. Such a system is able to analyze a smaller sample than would otherwise be required in the absence of the insert placed in the sample container.

[0030] Fig. 3B provides an illustration of a system including an analysis device, a sample container, and an insert configured to fit within the analysis device, in place of a sample container. Such a system is able to analyze a smaller sample than it would otherwise be able to do in the absence of the insert, which is used to hold the sample during analysis instead of the sample container.

DETAILED DESCRIPTION

[0031] Description and disclosure of examples of reagents, assays, methods, kits, devices, and systems which may be used with the methods, assays, reagents, devices and systems disclosed herein may be found, for example, in U.S. Patent Application Serial No. 61/858,589 filed July 25, 2013; U.S. Patent 8,380,541; U.S. Patent 8,088,593; U.S. Patent 8,380,541; U.S. Pat. App. Ser. No. 13/769,798, filed February 18, 2013; U.S. Pat. App. Ser. No. 13/769,779, filed February 18, 2013; PCT/US2012/57155, filed September 25, 2012; U.S. Patent Application

13/244,949, filed September 26, 2011; U.S. Application Serial No. 61/800,606, filed March 15, 2013; U.S. Application Serial No. 61/766,095, filed February 18, 2013; and U.S. Application Serial No. 61/673,245, filed September 26, 2011, the disclosures of which patents and patent applications are all hereby incorporated by reference in their entireties.

[0032] **DEFINITIONS**

[0033] As used herein, the term “original volume” and grammatical variants thereof refer to the sample volume used in an assay, or in an assay device, prior to modification or alteration of the assay method, or assay device.

[0034] As used herein, the term “reduced volume” and grammatical variants thereof refer to the sample volume used in an assay, or in an assay device, following modification or alteration of the assay method, or assay device, where such modification and alteration reduce the sample volume as compared to the original volume.

[0035] The word "label" or “marker” or the phrases “detectable label” and “marker moiety” when used herein refer to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. A label may be, without limitation, a dye, an epitope tag, a fluorescent moiety, a luminescent moiety, a chemiluminescent moiety, an enzymatic label, a magnetic label, a paramagnetic label, a contrast agent, a nanoparticle, a radioisotope, biotin, streptavidin, and a quencher.

[0036] A label may be an alkaline phosphatase label, in which the results of a reaction catalyzed by alkaline phosphatase is observed, and may be used to identify an analyte or verify its presence in a sample, and may be used to quantify an analyte in a sample. Alkaline phosphatase reagents are commercially available; for example, Nitroblue Tetrazolium (NBT) is used with the alkaline phosphatase substrate 5-Bromo- 4-Chloro-3-Indolyl Phosphate (BCIP) to provide a colored product which may be observed and quantitated. Other reagents include Fast Red TR/Naphthol AS-MX and TR phosphate (4-Chloro-2-methylbenzenediazonium/ 3-Hydroxy-2-naphthoic acid 2,4-dimethylanilide phosphate, reagents for the production of p-nitrophenol, and others.

[0037] For example, a label may be a peroxidase label (such as horseradish peroxidase, myeloperoxidase, or other peroxidase) in which the results of a reaction catalyzed by the peroxidase is observed, and may be used to identify an analyte or verify its presence in a sample, and may be used to quantify an analyte in a sample. Benzidine-containing compounds (e.g., diaminobenzidine, tetramethyl benzidine), aniline-containing compounds, aminoantipyrene compounds, Trinder reagents, and other reagents known in the art may be used to provide a detectable product in the presence of a peroxidase.

[0038] A label may be a dye, such as rhodamine and related rhodamine dyes (e.g., tetramethylrhodamine (TMR), carboxytatramethyl rhodamine (TAMRA), and others), fluorescein and fluorescein derivatives (e.g., 5-carboxyfluorescein, 6-carboxy fluorescein and others), phycoerythrin, umbelliferone, Texas Red, rare earth chelates (europium chelates), dansyl dyes (including, e.g., dansylamide dyes, dansyl cadaverine, dansyl chloride, and others); cyanine dyes (e.g., Cy3, Cy5, SYBR green, and others); Lissamine; phycoerythrins; Texas Red; and analogs thereof.

[0039] A label may be a fluorescent material, including fluorescent dyes, and including green fluorescent protein and other fluorescent proteins known in the art. A label may be a luminescent moiety, such as luminol, or other luminescent material, including bioluminescent materials such as luciferase, luciferin, and aequorin.

[0040] A label may be a nanoparticle, such as a gold nanoparticle (e.g., a colloidal gold particle), or a quantum dot (e.g., a small particle, typically a semiconductor, which may be detectable upon application of an appropriate amount and wavelength of electromagnetic radiation, e.g., by illumination). A label may be a magnetic label, or a paramagnetic label, which may be a nanoparticle or bead. A label may be a radioisotope or other radioactive material, including, e.g., ^{131}I, ^{125}I, ^{111}In, ^{99}Tc, ^{35}S, ^{14}C, and ^{3}H.

[0041] The term "quench" or "quenching" is used to indicate a reduction in detectable emission radiation, e.g., fluorescent or luminescent radiation, from a source that would otherwise have emitted this radiation. Quenching is a reduction of at least 50%, preferably 80% and more preferably 90%, of the detectable radiation from the source.

[0042] The term "quenchable dye" as used herein is a single molecular species that emits detectable radiation when in solution or bound to a single-stranded oligomer, either directly or through a linking moiety. The detectable radiation of a quenchable dye bound directly to a

single-stranded oligomer is reversibly quenched upon hybridization of the oligomer to a complementary oligonucleotide to form a hybrid duplex or triplex. No additional molecular species, e.g., a quenching dye, is required for the quenching to occur. However, if the quenchable dye is bound to the oligomer through a linker moiety, hybridization of the oligomer to its complement will not result in quenching of the detectable radiation emitted by the dye.

[0043] Fluorescent dyes that intercalculate with double-stranded DNA include, for example, SYBR Gold™, SYBR Green I™, SYBR Green II™, ethidium bromide, BlueView™, methylene blue, DAPI, DRAQ5 and related dyes, and acridine orange. Other fluorophores include, but are not limited to 7-dimethylaminocoumarin-3-carboxylic acid, dansyl chloride, nitrobenzodiazolamine (NBD), dabsyl chloride, cinnamic acid, fluorescein carboxylic acid, Nile Blue, tetramethylcarboxyrhodamine, tetraethylsulfohodamine, 5-carboxy-X-rhodamine (5-ROX), and 6-carboxy-X-rhodamine (6-ROX). Quenchers that may be used include, for example, DDQ-I, DDQ-II (Eurogentec), Eclipse (Epoch Biosciences), Iowa Black FQ, Iowa Black RQ (Integrated DNA Technologies), BHQ-1, BHQ-2, BHQ-3 (Biosearch Technologies), QSY-7, QSY-21 (Molecular Probes), and Dabcyl.

[0044] Dyes include, for example, CAL Fluor Gold, CAL Fluor Orange, Quasar 570, CAL Fluor Red 590, CAL Fluor Red 610, CAL Fluor Red 610, CAL Fluor Red 635, Quasar 670 (Biosearch Technologies), VIC, NED (Life Technologies), Cy3, Cy5, Cy5.5 (GE Healthcare Life Sciences), Oyster 556, Oyster 645 (Integrated DNA Technologies), LC red 610, LC red 610, LC red 640, LC red 670, LC red 705 (Roche Applies Science), Texas red, FAM, TET, HEX, JOE, TMR, and ROX. Non-limiting examples of near infrared dyes that can be conjugated to the antibodies, fragments, and/or derivatives of the presently disclosed subject matter include NIR641, NIR664, NIT7000, and NIT782. fluorescent label including, but not limited to Cy3, Cy5, Cy7, and any of the ALEXA FLUOR® series of fluorescent labels.

[0045] As used herein, the term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity. Thus, antibodies may be polyclonal antibodies, e.g., may be antibodies purified from the blood of an animal such as a sheep or goat which has been challenged by a target antigen, and may be monoclonal antibodies. For example, monoclonal antibodies may be made by the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No.

4,816,567 to Cabilly et al.). The "monoclonal antibodies" also include clones of antigen-recognition and binding-site containing antibody fragments (Fv clones) isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991), for example. Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

[0046] Antibodies (e.g., IgG antibodies) are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains (LCs) and two identical heavy chains (HCs). Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges between cysteines. Each heavy chain has at a variable domain, followed by a number of constant domains. The variable domains are disposed closer to the amino-terminal (N-terminal) portion of the HC than are the constant domains; conversely, the constant domains are disposed closer to the carboxy-terminal (C-terminal) portion of the HC than are the variable domains. Similarly, each light chain has a variable domain at one end (towards the N-terminal) and a constant domain at its other end (towards the C-terminal); the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia et al., J. Mol. Biol. 186:651 (1985); Novotny and Haber, Proc. Natl. Acad. Sci. U.S.A. 82:4592 (1985)). The variable domains form the antigen-binding sites; thus an intact antibody has two antigen binding sites composed of variable domains of the LC and HC pairs.

[0047] "Antibody fragment", and all grammatical variants thereof, as used herein are defined as a (1) portion of an intact antibody comprising the antigen binding site or variable region of the intact antibody, wherein the portion is free of the constant heavy chain domains (i.e. CH2, CH3, and CH4, depending on antibody isotype) of the Fc region of the intact antibody, and (2) constructs comprising a portion of an intact antibody (as defined by the amino acid sequence of the intact antibody) comprising the antigen binding site or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, $F(ab')_2$, Fd, Fc, Fv,

diabodies, and any other "Non-single-chain antigen-binding unit" as described, e.g., in U.S. Pat. No. 7429652. The term "intact antibody" refers to the complete antibody, or the amino acid sequence of the complete antibody, of which an antibody fragment is a part. It will be understood that an antibody fragment may be produced by partial digestion (e.g., by papain or pepsin) of an intact antibody, or may be produced by recombinant or other means.

[0048] As used herein, a "labeled antibody" refers to an antibody (whether intact or an antibody fragment) which is detectable by way of a label attached to the antibody. Such a label may be covalently attached to the antibody; such a label may be a dye, a radioisotope, a recognizable epitope (e.g., an epitope tag), or other label. A labeled antibody may include one, or may include multiple labels; multiple labels may be homogeneous or may be heterogeneous (e.g., a labeled antibody may have a plurality of fluorescent moieties covalently linked to the antibody (homogeneous) or may have a fluorescent moiety and a radioactive moiety linked to the antibody (heterogeneous)).

[0049] As used herein, a "finger-stick" refers to: i) the act of making a small puncture in the skin of a subject, allowing a small amount (e.g., a droplet, or one, two, or a few drops) of blood to flow and become available for collection; ii) the puncture itself; and iii) the blood collected thereby. Blood may be liberated in a finger-stick, for example, by use of a lancet or other sharp implement effective to pierce the skin of a subject. Typically, only a small amount of blood is collected in this way (e.g., the amount of blood may be about 250 μL or less, or about 200 μL or less, or about 150 μL or less, or about 100 μL or less, or about 50 μL or less, or about 25 μL or less, or about 15 μL or less, or about 10 μL or less, or about 10 μL or less, or about 5 μL or less, or about 3 μL or less, or about 1 μL or less). Blood from a finger-stick may be collected, e.g., by needle, syringe, capillary tube, or other method. Blood from a finger-stick may be collected for transport to another location; for storage prior to use or analysis; for immediate use; or for a combination of the same.

[0050] Typical assays of biological materials may require many milliliters of sample. In many cases where different assays are to be run, e.g., for multiple analytes, or for different analyte types, multiple samples may be obtained. Such volumes of sample, and such multiple acquisitions of sample from a subject, may be difficult, uncomfortable, and time-consuming.

[0051] However, reducing the volume of sample that is required for sample analysis provides advantages in comfort, accessibility, and cost; and may provide advantages in speed,

simplicity and ease of analysis. Increasing the speed of an assay provides quicker results, which will typically be appreciated by the subject from whom the sample is taken.

[0052] Some analysis devices and systems are described in the scientific literature, in some patents, and some analyzers are commercially available. Current commercial analyzers include the DiaSorin Analyzers (DiaSorin S. p. A., Saluggia, Italy); the ADVIA Chemical Systems (Siemens Healthcare Diagnostics, Malvern, Pennsylvania, USA); the BD Max™ (Becton Dickinson, Franklin Lakes, New Jersey, USA); the ThunderBolt® from Gold Standard Diagnostics (Davis, CA, USA); Cobas® Analyzers (Roche Diagnostics, Basel, CH); Ventana Symphony systems (Ventana Medical Systems, a division of Roche); the Bloodhound™ system of analyzers (Constitution Medical Investors, Inc., now a subsidiary of Roche); the CELL-DYN Ruby system (Abbott Diagnostics, Lake Forest, Illinois, USA); and others.

[0053] Current commercial analyzers typically add sample to a vessel (such as a cuvette) for placement in an analysis device or system. Reagents may be added to the sample, including, for example, reagents containing molecules which specifically bind to or react with a target substance, or analyte, in the sample. Further reagents may be added which label, or allow visualization or detection of, the target substance. The presence of the substance, or the amount of the substance, in the sample may be detected or measured. The detection or measurement may be compared to a calibration curve. Finally, the results of these actions may be printed out or otherwise communicated to a user.

[0054] As disclosed herein, it is advantageous to reduce the volume of sample required for testing. However, simply providing smaller amounts of sample to a machine, or using smaller amounts of sample in an assay, without further modifications, is typically fruitless, since the volumes of reagents; the concentrations of reagents and of constituents of reagents; the fluid-handling apparatus (where the sample is liquid); the transport means (for solid or fluid samples); the illumination means (if any); the signal detection apparatus; and other means, mechanisms, devices, systems, and system particulars may be incorrect, mis-matched, or incompatible with the reduced volume samples.

[0055] It is also advantageous to reduce the time required to perform a test. However, reducing the volume of a sample taken from a subject will reduce the amount of target substance in the sample, and will typically reduce the signal produced by assays for the detection or

quantification of the substance, all of which may lead to an increase in the time require to perform a test.

[0056] Applicants provide herein methods for reducing the volume of sample required for assays for detecting the presence of a target substance in a sample. Applicants provide herein methods for reducing the time required for the performance of assays for detecting the presence of a target substance in a sample. Applicants provide herein methods for reducing the volume of sample required and for reducing the time required for the performance of assays for detecting the presence of a target substance in a sample.

[0057] Applicants provide herein methods for reducing the volume of sample required for assays for quantifying the amount of a target substance in a sample. Applicants provide herein methods for reducing the time required for the performance of assays for quantifying the amount of a target substance in a sample. Applicants provide herein methods for reducing the volume of sample required and for reducing the time required for the performance of assays for quantifying the amount of a target substance in a sample.

Reducing the Volume of Sample Required to Perform an Assay

[0058] Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a sample container (such as, e.g., a cuvette, tube, caplet, or other container) is altered for use with smaller volumes of sample; such alterations include alterations in the volume of an internal chamber of a sample container (e.g., reduction in such volume), alterations in the shape of an internal chamber of a sample container (e.g., alterations making the chamber longer and narrower), alterations in a wall of an internal chamber of a sample container (e.g., providing a reflective, e.g., mirrored, or refractive (e.g., focusing) surface, and other alterations.

[0059] Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which an insert is provided and placed within a sample container (such as, e.g., a cuvette, tube, caplet, or other container), reducing the internal volume available to hold a sample. Such an insert may be configured to fit snugly within a cavity of a sample

container, and to have a cavity for holding a sample, where the cavity of the insert has a smaller volume than the cavity of the sample container.

[0060] Devices useful for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include inserts configured for placement within a sample container having a sample container cavity (such as, e.g., a cuvette, tube, caplet, or other container), the insert having an internal cavity whose volume is less than the volume of the sample container cavity.

[0061] Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a detector, such as a light detector, or an electronic detector, or other detector, is altered for use with smaller volumes of sample. Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a sample container is altered for use with smaller volumes of sample, so that, for example, a long narrow chamber provides a suitable pathlength for optical detection while requiring a smaller sample volume than an original container. Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a source of illumination required for optical detection or measurement is altered for use with smaller volumes of sample; typically, such alteration includes increasing illumination intensity, but may also, or instead, include altering the wavelength of light, or the polarization of light, provided by the source of illumination. Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a sample is placed nearer to a detector, such as a light detector, or an electronic detector, or other detector, so that a smaller signal from a smaller sample volumes remains detectable. Thus, alterations suitable for use with smaller sample volumes include increasing the sensitivity of the light detector, or electronic detector; increasing the intensity of illumination used in analysis of smaller volumes of samples; increasing the path length through which light passes prior to detection by a detector; decreasing the distance between the sample and a detector; altering the wavelength of light, or detecting multiple wavelengths of light, passing thorough or absorbed by a smaller volume of sample; and other alterations.

[0062] Methods for reducing the volume of a sample used during sample analysis, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a small sample is diluted with a diluent, such as water, or buffer, or other diluent. In this way, the volume of the material presented for analysis and detection may remain the same, or may be similar, to the volume that was initially used or required. Since a diluted sample may provide a smaller signal than an undiluted sample, similar modifications in light intensity, illumination wavelength, container configuration, container volume, sample placement, and other modifications may be used with a diluted sample.

[0063] Methods for reducing the volume of a sample used during sample analysis, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which a larger amount (absolute amount) of dye, or enzyme substrate, or other molecule used in producing a signal indicative of the presence, or amount, of a target molecule, is added to a small sample; or methods in which a larger concentration of dye, or enzyme substrate, or other molecule used in producing a signal indicative of the presence, or amount, of a target molecule, is added to a small sample; or a dye, enzyme substrate, or other molecule used in producing a signal indicative of the presence, or amount, of a target molecule, providing a more intense or stronger signal is added to a small sample, as compared to the original dye amount, concentration, or make-up.

[0064] Methods for reducing the volume of a sample, e.g., reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include methods in which the optical properties of a sample container are altered so as to focus, or reflect, light impinging on the sample to provide increased light intensity to the sample (e.g., increased light intensity to a smaller volume, where the sample is a smaller volume sample) as compared to the optical properties and volumes of the original configuration. Thus, a sample container may be altered to include lenses, or lensing surfaces, which refract light onto a sample chamber, or onto a portion of a sample chamber, in which a sample is held. Thus, a sample container may be altered to include mirrors, or mirrored surfaces, which reflect light onto a sample chamber, or onto a portion of a sample chamber, in which a sample is held.

[0065] Accordingly, methods for reducing the amount of sample required for an assay for the detection of, or quantification of, a target substance in a sample, include the following methods.

[0066] Applicants disclose methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, where the assay comprises optical detection of a target substance in a sample. Such methods may comprise, for example, reducing the volume of sample used in the assay from a first (larger) volume to a second (smaller) volume, where the assay was previously performed using a first volume of sample; and increasing the intensity of illumination applied to the sample. Such increased illumination is used to detect or quantify a target substance in the sample. Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since many optical signals are enhanced at higher levels of illumination intensity. Such detection, quantification, or detection and quantification may include absorbance measurements, transmission measurements, turbidity measurements, polarization measurements, circular dichroism measurements, light scattering measurements, and other optical measurements. It will be understood that these, and other optical measurements, may be used for any of the methods, such as, e.g., any optical methods, disclosed herein.

[0067] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, include methods where the assay comprises detection of a fluorescent label. Such methods may comprise, for example, reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the intensity of light illuminating the sample and assay reagents during fluorescence measurements. Such methods may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since fluorescence from many labels is enhanced at higher levels of illumination.

[0068] Thus, as disclosed herein, an increase in light intensity may be utilized in methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample. Light intensity may be increased from a light

source by any suitable means. Providing an additional light source, or a plurality of additional light sources, is effective to increase the intensity of illumination applied to a sample. Increasing the power (e.g., by increasing the current, or by increasing the voltage, or both) applied to a light source is effective to increase the intensity of illumination applied to a sample. Providing a different light source, capable of providing increased light intensity, is effective to increase the intensity of illumination applied to a sample; for example, an original light source of a first type may be replaced by, e.g., an incandescent light source, a halogen light source, a laser light source, a diode light source, a sodium vapor light source, or other light source. Lenses, mirrors, prisms, fiber optics, and other optical elements may be provided effective to increase the intensity of illumination applied to a sample.

[0069] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, comprise assays comprising detection of a dye. Such methods, for example, may comprise reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the concentration of a dye added to the sample, where the dye labels a target substance in said sample. Such methods may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since signals from many dyes or other labels are enhanced at higher concentrations.

[0070] In embodiments, methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, may comprise detection of a dye. Such methods, for example, may comprise reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the amount of a dye added to the sample, where the dye labels a target substance in said sample. Such methods may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since signals from many dyes or other labels are enhanced with greater amounts of dye.

[0071] Thus, as disclosed herein, dyes may be utilized in methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a

target substance in a sample. Dyes suitable for use in the methods disclosed herein include fluorescent dyes, chemiluminescent dyes, nuclear dyes, membrane dyes, Nile Blue, Coomassie blue, rhodamine dyes, fluorescein dyes, Sybr dyes, DRAQ5 and related dyes, fluorescent proteins (e.g., green fluorescent protein and related proteins), resazurin (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide); 10-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) and similar compounds (e.g., Amplex UltraRed (A36006 from Life Technologies, Carlsbad, CA 92008); resorufin compounds (e.g., 7-ethoxyresorufin); dyes such as e.g., fluorescein, calcein, rhodamine, and ethidium dyes; *N*-methyl-4-hydrazino-7-nitrobenzofurazan; acridinium (acridine-9-carboxylic acid) esters and compounds which react with these compounds to alter an optical property of a solution; phenols and phenol derivatives (e.g., p-iodophenol and p-phenylphenol); luminescent amines, including amine adducts (e.g., as may be derived from copper cyanide), silver stain, and other dyes and stains as disclosed herein and as known and used in the art.

[0072] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, may comprise detection of an enzymatic label. Such methods, for example, may comprise reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the concentration of substrate added in the presence of the enzyme prior to, or during enzymatic label measurements. Such methods may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since signals from many enzymatic labels are enhanced at higher levels of substrate concentration.

[0073] Thus, as disclosed herein, enzymatic labels and substrates may be utilized in methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample. Enzymes and substrates suitable for use in the methods disclosed herein include horseradish peroxidase (the enzyme) and peroxide (the substrate). Although horseradish peroxidase is specifically recited herein, any peroxidase (and substrates of the peroxidase) that participates in a reaction with its substrate(s) to form a colored product may be used in the detection and/or quantification of an analyte in a sample. For example, the colorant horseradish peroxidase (HRP) participates in a reaction with any one or more of several molecules effective to change the optical properties of a solution to which the

HRP is added (e.g., by changing the color, the absorbance of light through a solution to which the HRP is added, and/or other optical properties of the solution). For example, HRP may react with an aniline-containing compound such as N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (ALPS), or with an aminoantipyrene compound such as 4-aminoantipyrene or with phenolic compounds. Thus, for example, a peroxidase (e.g., HRP, myeloperoxidase, or other peroxidase), an aniline-containing compound, and an aminoantipyrene may all be termed "colorants." In further examples, HRP may react with a benzidine-containing compound (e.g., with diaminobenzidine (DAB); tetramethylbenzidine(TMB); 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (DABS); 3-dimethylaminobenzoic acid (DMAB); hydroquinone; o-tolidine; o-phenylenediamine; o-chlorophenol; p-hydroxy-benzenesulfonate; p-anisidine; a Trinder reagent (such as 4-aminoantipyrene, methylbenzothiazolinonehydrazone (MBTH), or other compound for producing a Trinder dye); and derivatives and related compounds) to form a colored product. HRP or other peroxidase may also react with other compounds to form a chemiluminescent product; for example, HRP or other peroxidase may react with luminol to form a chemiluminescent product (other molecules may be present, and may enhance such reactions; for example, HRP-mediated production of luminescent products from luminol is enhanced in the presence of 4-iodophenol). It will be understood that other enzymes and reactants may be used to form colored products useful for the detection of an analyte in a sample.

[0074] Alkaline phosphatase reagents are commercially available; for example, Nitroblue Tetrazolium (NBT) is used with the alkaline phosphatase substrate 5-Bromo- 4-Chloro-3-Indolyl Phosphate (BCIP) to provide a colored product which may be observed and quantitated. Other reagents include Fast Red TR/Naphthol AS-MX and TR phosphate (4-Chloro-2-methylbenzenediazonium/ 3- Hydroxy-2-naphthoic acid 2,4-dimethylanilide phosphate, reagents for the production of p-nitrophenol, and others known in the art may be used.

[0075] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, include methods where the assay comprises labeled antibody detection of a target substance. Such methods comprise, for example, reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the concentration of labeled

antibody, or increasing the number of labels per antibody, or altering the label to provide a more detectable signal. Such methods may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since signals from more, or more highly labeled, or more detectably labeled antibodies are enhanced as compared to signals provided by the original methods.

[0076] Methods for labeling antibodies are known in the art, including methods for providing multiple labels, or different labels, on antibodies. Methods of conjugating labels and other moieties to antibodies and other proteins are discussed, for example, in Wofsy et al. Selected Methods in Cellular Immunology, p. 287, Mishel and Schiigi (eds.) San Francisco: W. J. Freeman Co. (1980)); Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., Immunol. Rev., 62:119-58 (1982).

[0077] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, include assays comprising detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with the target substance. Such methods comprise, for example, reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the sensitivity of an optical detector used to detect a signal indicative of the presence or, or amount of, target substance in the sample. Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since increased sensitivity to signals indicative of the presence or, or amount of, target substance in a sample will allow detection and quantification from smaller or less intense signals, such as those signals expected from smaller volume samples.

[0078] The sensitivity of a detector may be increased by providing additional sensors or detectors; by replacing a less-sensitive detector or sensor with a more-sensitive detector or sensor; by providing lenses, mirrors, or other optical enhancements which collect or focus a signal and direct it to the sensor or detector; by reducing "noise" in the sensor or detector (e.g., by cooling the sensor or detector, or by filtering the power driving the sensor or detector, or by other means); and by other means.

[0079] A related method for reducing the volume of sample used in the performance of an assay for the optical detection of, or optical quantification of, a target substance in a sample, comprises reducing the volume of sample, and decreasing the separation between the sample and an optical detector used to detect a signal indicative of the presence or, or amount of, target substance in the sample. Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since such a reduced separation allows detection smaller signals indicative of the presence or, or amount of, target substance in a sample, such as those signals expected from smaller volume samples.

[0080] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, include methods where the assay comprises detection of optical absorbance produced by a target substance, or by a target substance and a reagent which binds to or reacts with the target substance. Such methods may comprise reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the path length within the sample between a source of illumination and through the sample to an optical detector used to detect an absorbance signal indicative of the presence or, or amount of, target substance in the sample. Increasing the optical path length through the sample provides a larger signal, since more sample is traversed by the light traveling along the longer path through the sample.

[0081] Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since such an increased path length through the sample would allow detection smaller signals indicative of the presence or, or amount of, target substance in a sample, such as those signals expected from smaller volume samples. In embodiments, such an increased path length may be provided, even for a

reduced volume sample, by, for example, providing a cuvette or other sample container having a long, narrow chamber, in which the sample resides and along the long portion of which the illumination is provided. Such a cuvette or sample container comprises an altered chamber dimension as compared to a cuvette or sample container as used in the assay for larger volume samples. In embodiments, such an increased path length may be provided, even for a reduced volume sample, by, for example, providing a mirror or mirrored surface at a position at or near a wall of the cuvette or other sample container, so that light from an illumination source follows a longer path, including a reflected portion, within the sample resides prior to detection of the light for an absorption measurement. Such a longer path for use with a reduced volume sample is longer as compared to a path for absorption measurements for larger volume samples.

[0082] Further methods for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, include methods where the assay comprises detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with the target substance. Such methods may comprise reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and altering the wavelength of light, or detecting multiple wavelengths of light, passing through or absorbed by a smaller volume of sample. Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since such an altered light wavelength, or such a plurality of wavelengths, allow detection smaller signals indicative of the presence or, or amount of, target substance in a sample, such as those signals expected from smaller volume samples.

[0083] Alteration of wavelengths of light may be accomplished by altering a light source; by passing light from a light source through a filter, or through a prism, or other optical element; by providing one or more additional light source(s); or any combination of these, among other methods which may be used to alter light wavelengths used to illuminate a sample.

[0084] Further methods for reducing the volume of sample used in the performance of an assay for the optical detection of, or optical quantification of, a target substance in a sample, include altering the polarization of light used to illuminate a sample. Such methods may comprise reducing the volume of sample used in the assay from a first volume to a second

volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and altering the polarization of light passing through or absorbed by a smaller volume of sample. Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since such altered light polarization allows detection signals indicative of the presence or, or amount of, target substance in a sample, such as those signals expected from smaller volume samples.

[0085] Altering the polarization of light may be accomplished, for example, by altering a light source; by passing light from a light source through a filter, or through a prism, or grating, or slit (or slits), or other optical element; by reflecting light from a surface; or any combination of these, among other methods which may be used to alter the polarization of light used to illuminate a sample.

[0086] Applicants further disclose a method for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, where the assay comprises detection of an electrical signal produced by a target substance, or by a reagent which binds to or reacts with the target substance, the method comprising:

[0087] Reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the electronic amplification of an electrical detector used to detect an electrical signal indicative of the presence or, or amount of, target substance in the sample. Such a method may allow detection, quantification, or detection and quantification of the target substance in a reduced volume of sample, since such an increased electronic amplification of such an electrical detector would allow detection smaller signals indicative of the presence or, or amount of, target substance in a sample, such as those signals expected from smaller volume samples. Electrical detectors include, without limitation, ion-sensitive electrodes, amperometric detectors, and voltammetric detectors. Such detectors provide electronic output, and amplification of signals from such detectors may be performed by standard electronic techniques and devices. Amperometric techniques are discussed, for example, in "Amperometric Techniques" by Thomas Roussel et al., in the Encyclopedia of

Microfluidics and Nanofluidics, pages 39-47, Springer Verlag (2008). Voltammetric techniques are discussed, for example, in Kounaves, "Voltammetric Techniques", Chapter 37 (pages 709-725) in: Handbook of Instrumental Techniques for Analytical Chemistry, edited by Frank Settle, Prentice-Hall (1997). Ion-sensitive electrodes are discussed, for example, in Primer: Ion selective measurement in online analysis, by YSI, a Xylem brand (2012) (available at: http://www.ysi.com/media/pdfs/ba76001-Online-ISE-Primer-e01.pdf).

[0088] A method for reducing the volume of sample used in the performance of an assay for the detection of, or quantification of, a target substance in a sample, the method comprising:

[0089] Reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, wherein the assay was previously performed using a first volume of sample; and increasing the temperature of the assay. Such a method may allow detection, quantification, or detection and quantification of the target substance in the reduced volume of sample, since many labels and labeling reactions are enhanced at higher temperatures than at lower temperatures. For example, where photodetection of an enzymatic label (e.g., alkaline phosphatase, horse radish peroxidase, or other enzymatic label) in which the rate of production of a detectable product may be temperature sensitive, increased signal may be produced by reaction at higher temperatures. For example, where an assay is performed at room temperature (e.g., near 20 °C), increasing the assay temperature to about 25 °C, or about 30 °C, or about 32 °C, or about 34 °C, or about 35 °C, or about 36 °C, or about 37 °C, or about 38 °C, or about 39 °C, or about 40 °C, or higher, may increase the signal produced by an otherwise unchanged reaction.

[0090] *Reducing the Time Required to Perform an Assay*

[0091] Reducing the amount of time required to perform an assay (while maintaining the accuracy or precision of the assay) allows for processing more samples in a unit time than would otherwise be possible. Methods for reducing assay duration are discussed, for example, in U.S. Patent Application 61/858,589, filed July 25, 2013, the contents of which is hereby incorporated by reference in its entirety.

[0092] Methods for reducing the amount of time required to perform an assay for the detection of, or quantification of, a target substance in a sample, include, but are not limited to, the following methods.

[0093] A method for reducing the time required for the performance of an assay for the detection of, or quantification of, a target substance in a sample, the method comprising:

[0094] Increasing the temperature of the assay.

[0095] A further method for reducing the time required for the performance of an assay for the detection of, or quantification of, a target substance in a sample, the method comprising:

[0096] Reducing the volume of sample used in the assay from a first volume to a second volume, wherein said second volume is less than said first volume, and wherein the assay was previously performed using a first volume of sample; and increasing the temperature of the assay.

[0097] Such methods may allow detection, quantification, or detection and quantification of the target substance in the sample, since many the rate of reactions producing labels and labeling reactions are increased at higher temperatures than at lower temperatures. For example, where photodetection of an enzymatic label (e.g., alkaline phosphatase, horse radish peroxidase, or other enzymatic label) in which the rate of production of a detectable product may be temperature sensitive, a detectable amount of signal may be produced more quickly at higher temperatures than at lower temperatures. For example, where an assay is performed at room temperature (e.g., near 20 °C), increasing the assay temperature to about 25 °C, or about 30 °C, or about 32 °C, or about 34 °C, or about 35 °C, or about 36 °C, or about 37 °C, or about 38 °C, or about 39 °C, or about 40 °C, or higher, may reduce the amount of time required to produce a detectable amount of the signal, as compared to the original (lower temperature) reaction.

[0098] *Devices for Reducing the Volume of a Sample (e.g., Inserts)*

[0099] Sample analysis devices, and sample analysis systems including sample analysis devices, typically have one or more sample containers which are configured to hold a sample during analysis. Applicants disclose herein devices, systems, and methods for reducing the volume of sample required for analysis.

[00100] Embodiments of these devices for reducing the amount of sample needed or used for sample analysis include inserts configured to fit inside a sample container, and to provide a chamber in which a sample – of smaller volume than the volume of sample held by the sample container – may be held during analysis by a sample analysis device or sample analysis system.

The sample container with an insert within it, and sample within the insert, may be used to perform analysis of the sample in the insert.

[00101] Embodiments of devices for reducing the amount of sample needed or used for sample analysis include inserts configured to replace a sample container, and to provide a smaller chamber than the one in the sample container. A sample – of smaller volume than the volume of sample held by the sample container – may be held in an insert that replaces a sample container during analysis by a sample analysis device or sample analysis system.

[00102] A sample container includes a cavity, or internal chamber, which may hold a sample. A sample container is typically designed so that fluid (e.g., a sample) held within the sample container is retained by gravity; that is, the wall or walls of the cavity are typically vertical, or nearly vertical, when the sample container is in use. A sample container has an opening through which a sample may be introduced into the bore of the sample container; the opening may define a plane, and the "width" the opening may be termed as being in an orientation that is substantially parallel to the plane of the opening. Thus, in order to introduce a sample into the cavity of a sample container, the sample must pass through the plane of the opening (e.g., along a path substantially perpendicular to the plane of the opening). The "depth" of the cavity of a sample container may be termed as being in an orientation substantially perpendicular to the plane of the opening.

[00103] A device placed within the cavity, or internal chamber, of a sample container reduces the volume within the sample container that is available to hold the sample. Thus, placing an object or device within a sample container reduces the effective volume of the sample container cavity and reduces the volume of sample that may be held within the sample container. An object of any shape able to fit within the sample container, while leaving some volume available to hold a sample, may be used to reduce the volume of sample used in the performance of an assay. In embodiments, such an object may be an insert configured to fit within a sample container, where the insert includes a cavity or chamber configured to hold a sample. Placement of such an insert is effective to reduce the internal volume available to hold a sample.

[00104] Accordingly, Applicants disclose herein designs and descriptions for inserts to be placed within a cavity of a sample container, thereby to reduce the effective volume of the cavity of a sample container, and thus to reduce the volume of sample required for the performance of

sample analysis. For example, an insert configured to fit within a sample container may fit snugly within the cavity, or chamber, of a sample container, leaving little or no space between the outer wall of the insert and the inner wall of the sample container's cavity or chamber. For example, such an insert may be configured to fit snugly within a sample container and have a cavity or chamber which is configured to hold a sample with little risk of loss due to leakage, or spillage. An insert fits within the cavity of a sample container. The wall or walls of such an insert define its cavity, termed an "insert cavity"; an insert cavity has a smaller volume than the cavity of the sample container. The walls of an insert, and an insert cavity within an insert, may be configured to allow transmission of light or other signals through the insert. In embodiments, an insert configured to allow transmission of light or other signals through the insert is configured to allow such transmission with little loss of light intensity, or with little distortion, or with little effect on light polarization, or combinations thereof.

[00105] An insert may be made from glass (e.g., borosilicate glass, aluminosilicate glass, quarts, or other glass), or a plastic or acrylic compound (e.g., polymethyl methacrylate, or other acrylics), or a polymer (such as, e.g., polypropylene, polystyrene, polycarbonate, polyurethane, and other polymers), or combinations of these, or other material or combinations of materials. Preferred materials, such as acrylic compounds, provide good fluid flow, enhancing recovery of fluid from the well of the insert.

[00106] An example of an insert configured to fit within a sample container is shown in Fig. 1. Insert 110 has an insert cavity 120 defined by a vertical wall 130 and a floor 140. The opening 150 of the insert cavity 120 allows sample to be placed in, or removed from, the insert 110. The insert 110 has an outer wall 160 and a base 170, both of which are configured to fit within a sample container. The shape and orientation of the floor 140 may be flat, or may be beveled, or may be rounded, or may be a combination of such shapes, or may be a complex or an irregular shape. The outer wall 160 of an insert 110 is configured to fit within the cavity of a sample container; such an outer wall 160 may be vertical (e.g., as shown in the example shown in Fig. 1), or may be at an angle; for example, an outer wall may be angled so that the outer diameter of an insert is greatest near the opening, and smallest near the floor, or near the base of the insert. In embodiments, an insert may have a substantially cylindrical shape (e.g., as indicated in Fig. 1); or may have a tapered, or partially conical shape (e.g., where the diameter

near the base is less than the diameter near the opening); or may have a square, or rectangular, or triangular, or other shape.

[00107] Reducing or eliminating the volume that is substantially inaccessible to a probe or sensor is an important consideration in the design of an insert. For example, some sample containers require there to be an amount of sample at the bottom of the container in order to fill the sample container up to a level accessible for measurements. It is desirable to eliminate or reduce such sample volume that is required – but not measured - before a measurement may be obtained. This may be done by altering the shape of the bore in which the sample is held. A bore may have a rounded (e.g., circular, or elliptical) horizontal cross-sectional shape; may have a square or rectangular horizontal cross-sectional shape; or other horizontal cross-sectional shape. Where a probe is to be placed within a bore of an insert, reducing the diameter or width of the bore to as closely approximate the diameter or width of the probe is useful to reduce the amount of inaccessible volume in the insert. Similarly, where a probe is to be placed within a bore of an insert, reducing the depth of the bore to as closely approximate the depth achieved by the probe within the bore is useful to reduce the amount of inaccessible volume in the insert.

[00108] Thus, the depth of an insert cavity is an important characteristic. The depth of an insert cavity, along with the width of an insert cavity, may be designed so as to reduce the total volume of the insert cavity, and also may be designed so as to reduce the volume of sample which may be inaccessible to a probe inserted into the insert cavity. Reducing the total volume of an insert cavity is a design goal for providing an insert suitable for reducing the amount of sample required for sample analysis. A further design goal for providing an insert suitable for reducing the amount of sample required for sample analysis is to reduce, or eliminate, the volume in the insert cavity that may be inaccessible to a probe placed in a sample in an insert cavity.

[00109] Accordingly, the shape of the floor of the insert cavity is a further consideration in the design of an insert. Providing a bevel at the base of the bore of an insert may aid in allowing a probe to reach its maximum depth within a bore, while reducing the amount of inaccessible volume within the bore. In addition, a bevel may aid in fluid flow (e.g., flow into, or out of the insert cavity, or both). In embodiments, such a bevel may include a conic (e.g., triangular cross-section) bevel; a circular or hemispherical (e.g., a rounded or partly circular cross-section) bevel;

an oval or elliptical (e.g., a rounded but not circular cross-section); or other shape. In embodiments, a bevel may include a combination of two or more such shapes (e.g., may include conic portions and may include circular portions; or may include flat portions and circular portions; or may include flat portions and conic portions).

[00110] An illustration of an exemplary insert having an insert cavity is shown in Fig. 2A. The perspective view of the insert 200 shows an opening 215 through which a sample may pass into the insert cavity 210 (shown in Fig. 2B). The insert 200 shown in Fig. 2A has an upper portion with an outer wall 250, a lower portion with an outer wall 255, and a lip 260. The exemplary insert 200 shown in Fig. 2A has a base 220 at its bottom. A lip 260 may be configured to lodge on a surface of a sample container when an insert 200 is placed within the sample container; thus, a lip 260 may help with the proper placement of an insert in a sample container, and so insure the proper operation of a sample analysis device or system in which an insert 200 is in place in a sample container. In embodiments, an insert 200 may have no lip 260, and the upper portion wall 250 may be flush with the lower portion wall 255. The walls 255 and 260 are shown as being vertical, i.e., perpendicular to the plane suggested by the opening 215. In embodiments, a lower portion wall 255 may be tapered, so that the insert 200 may be wider nearer the opening 215 than it is nearer the base 220. A wall 250 or 255, or both, may have other shapes and orientations as well; for example, an upper portion wall 250 may have a substantially vertical orientation (e.g., similar to that shown in Fig. 2A) while a lower portion wall 255 may be angled so as to taper from a wider to narrower along the wall in the upper to lower direction. In embodiments, an upper portion wall 250 may be tapered, while a lower portion wall 255 may have a substantially vertical orientation (e.g., similar to that shown in Fig. 2A). In embodiments, both an upper portion wall 250 and a lower portion wall 255 may be angled so as to taper; the upper taper may be the same as the lower taper, or the upper taper may be different than the lower taper. In embodiments, such tapers may become narrower towards the lower portion; in embodiments, such tapers may become wider towards the upper portion.

[00111] An illustration of an exemplary bevel in the bottom portion of an insert cavity is shown in Fig. 2B, which provides a cross-section of an insert 200 having features as disclosed herein. In the example shown, the floor 230 of the cavity 210 is flat, and has a flat bevel wall 235 disposed at an angle to the vertical inner wall 225 of the cavity 210. As shown, the junctions between the flat portions are rounded. In embodiments, the junctions between flat portions may

not be rounded. As shown, the bottom portion of the insert cavity 210 includes a flat portion (floor 230) and rounded portions (wall angle 240 and floor angle 245). Wall angle 240 is the angle between inner wall 225 and bevel wall 235; floor angle 245 is the angle between bevel wall 235 and floor 230. In the example shown, floor 230 is perpendicular to inner wall 225, so that wall angle 240 and floor angle 245 are complementary. Thus, in the example shown in Fig. 2B, floor angle 245 is about 30° and wall angle 240 is about 60°. In embodiments of inserts having features as disclosed herein, floor angle 245 may be between about 10° to about 50°and wall angle 240 may be between about 80° to about 40°. In further embodiments of inserts having features as disclosed herein, floor angle 245 may be between about 25° to about 45°and wall angle 240 may be between about 65° to about 45°. In further embodiments, an inner wall 225 is not perpendicular to floor 230; in these embodiments, wall angle 240 and floor angle 245 will not be complementary. In embodiments, wall 225 need not be vertical; for example, it may be angled so that the width 265 of the cavity 210 is greater near the opening 215 than it is near to the floor 230. In the example shown in Fig. 2B, the depth 270 of cavity 210 is about the same as the width 265. In embodiments, the ratio of depth 270 to width 265 may be between about 0.1:1 to about 10:1 (i.e., from a ratio in which depth 270 is about one tenth the width 265, to a ratio in which depth 270 is about ten times the width 265). In embodiments, the ratio of depth 270 to width 265 may be between about 0.2:1 to about 5:1 (i.e., from a ratio in which depth 270 is about one fifth the width 265, to a ratio in which depth 270 is about five times the width 265). In embodiments, the ratio of depth 270 to width 265 may be between about 0.5:1 to about 3:1 (i.e., from a ratio in which depth 270 is about half the width 265, to a ratio in which depth 270 is about 3 times the width 265). In embodiments, the ratio of depth 270 to width 265 may be about 0.3:1; or about 0.5:1; or about 0.8:1; or about 1:1 (e.g., similar to that shown in Fig. 2B); or about 1.2:1; or about 1.5:1; or about 1.8:1; or about 2:1; or about 3:1; or about 4:1; or about 5:1; or about 6:1; or about 8:1; or other ratio.

[00112] In further embodiments, an insert may be configured to completely replace an original sample container. In such embodiments of devices for reducing the amount of sample needed or used for sample analysis, an inserts may be configured to replace a sample container by having the same, or compatible outside dimensions as the sample container, and having a insert cavity as disclosed herein. The insert cavity of an insert configured to replace a sample container may be the same as an insert configured to fit within a sample container. Thus, in

embodiments, an insert configured to replace a sample container may have an insert cavity having the same dimensions, including the same ratio of depth to width, and the same taper, and the same bevel, as disclosed herein with respect to inserts configured to be placed within a sample container. A an insert configured to replace a sample container may be made from the same materials as disclosed herein as being suitable for inserts configured to be placed within a sample container.

[00113] Accordingly, inserts configured to be placed within a cavity of a sample container, or replacement inserts configured to substitute for a sample container, are effective to provide a smaller volume chamber for holding sample during analysis. Such inserts are effective to reduce the volume of sample required for the performance of sample analysis.

[00114] *Kits for Reducing the Volume of a Sample*

[00115] A device, or devices, which may be inserted into a sample container may be provided as part of a kit; such a kit may include instructions for the use of the inserts, including instructions for placement of the inserts into a sample container, instructions for cleaning an insert, and other instructions. A kit may include materials for maintaining, including materials for cleaning, an insert. A kit may include tools useful for placing an insert into a sample container, or for removing an insert from a sample container. A kit may include materials useful for securing, or for the secure placement of an insert into a sample container, or for insuring that an insert remains in place within a sample container after placement. A kit may include reagents for use with the inserts and with a sample container.

[00116] For example, instructions for the use of the inserts may include such information as that it may be desirable to insure that an insert is fully in place within a sample container before use; or that it may be desirable to clean (e.g., rinse with detergent, or solvent, followed by a rinse with water, such as de-ionized water; or to a rinse with water without use of detergents or solvents) prior to placement within a sample container; and other instructions. A kit may include instructions for the proper handling of an insert (e.g., use of gloves to avoid contamination during placement) of an insert into a sample container.

[00117] A kit may include materials for maintaining, including materials for cleaning, an insert. For example, where the insert may be cleaned with a detergent or solvent, the detergent or solvent may be provided as part of a kit. Water, such as de-ionized water, may be provided as

part of a kit. A kit may include tools useful for handling an insert; for example, tongs or clamps (which may include, e.g., elongated handles for ease of placement of an insert in a location that is otherwise difficult to access by hand). A kit may include clamps, or tape, or shims, or other materials which may be useful for securing an insert in a sample container. A kit may include reagents (e.g., diluents, reactants, or other reagents) for use with the inserts and with a sample container.

[00118] *Systems for Reducing the Volume of a Sample*

[00119] Systems for reducing the volume of sample required for analytical measurements include an analysis device, a sample container, and an insert for placement within a sample container. As discussed above, a device as disclosed herein may be provided for placement within the cavity, or chamber, of a sample container, effective to reduce the volume within the sample container. The sample container, with insert, may be placed within an analysis device, and a sample placed in the sample container (i.e., within the insert disposed within the sample container) and the sample analyzed. In this way, a system is provided effective to analyze a sample, where the volume of the sample required or analysis is reduced as compared to the volume required by the analysis device in the absence of the insert, and in the absence of the use of methods disclosed herein. Such an insert, as disclosed herein, reduces the volume of the sample container and reduces the volume of sample that may be held within the sample container. Thus, systems comprising an insert (including any insert of any shape suitable to fit within a sample container, while leaving some volume available to hold a sample), may be used to reduce the volume of sample used in the performance of an assay.

[00120] Fig. 3A provides an illustration of a system including an analysis device, a sample container, and an insert configured to fit within the sample container. An analysis system 300 is shown, including an analysis device 310, which uses a sample container 320 having outer wall 330 to hold a sample during analysis. Sample container 320 has a cavity 340 defined by an inner wall of the sample container (shown with dashed lines). Fitted within the sample container cavity 340 is an insert 350; the insert 350 has an insert cavity 360 (shown with dotted lines).

[00121] The sample container 320 (with the insert 350 in place within the cavity 340 of the sample container) is in place within the analysis device 310, effective that a sample may be analyzed by the analysis device 310. However, with the insert 350 in place within the cavity 340

of the sample container 320, the volume of sample that is analyzed is less than the volume of sample that would have been required if the insert 350 had not been placed within the cavity 340 of the sample container 320.

[00122] An insert is thus configured to reduce the sample volume required for sample analysis by an analysis device or an analysis system. In embodiments, an insert has features as disclosed herein, and is configured to enhance fluid flow in the insert cavity. In embodiments, an insert has features as disclosed herein, and is configured both to reduce the sample volume required for sample analysis by an analysis device or analysis system, and to enhance fluid flow in the insert cavity.

[00123] Fig. 3B provides an illustration of an analysis system 300 including an analysis device 310, but without a sample container. In place of a sample container, a sample is held by an insert 350 configured to replace the sample container; analysis by sample analysis device 310 proceeds with the sample held in insert cavity 360 (shown with dotted lines) within the insert 350. The outer wall 370 of the insert 350 that replaces a sample container has dimensions and shape that is compatible with the analysis device 310; thus, outer wall 370 of the insert 350 is, as far as is necessary to operably fit in analysis device 310, the same as outer wall 330 of the sample container as shown in Fig. 3A.

[00124] Insert 350, positioned in place of a sample container within the analysis device 310, is effective to hold a small volume of sample for analysis by analysis device 310. The volume of sample that is analyzed in this way is less than the volume of sample that would have been required if the insert 350 had not been used in place of the sample container (e.g., sample container 320).

[00125] Applicants have performed experiments using commercial analysis devices by modifying some aspects of the hardware and some aspects of the software in order to customize assays used to analyze blood samples. General approaches used in these modifications include, for example, reducing the final volume read by the detector; optimization of the dilution levels for each assay; not using the ion selective electrode detectors; optimizing the chloride content of reagents and optimizing the protocols which use chloride-containing reagents; modifying the design of the sample container to reduce sample volume and to reduce overage (e.g., reduce the risk of spills or wasted sample); including a predilution step in order to predilute the plasma in

the sample containers; selecting which assays should be done in EDTA-containing solutions and which in heparin-containing solutions in order to meet the assay requirements while at the same time also minimizing sample volume required for each assay. Such modifications were aimed at optimizing assay protocols so as to minimize overages in dilution vessels and in pipetting operations.

DEVICES, METHODS AND SYSTEMS FOR REDUCING SAMPLE VOLUME

WHAT IS CLAIMED IS:

1. A method of analyzing a sample with a sample analysis device having a sample container, wherein said sample is contained within said sample container during at least a portion of the performance of said sample analysis, said sample container comprising an internal cavity for holding said sample, said internal cavity having a volume, said internal cavity volume of said sample container comprising an original volume, a volume of sample being held within said internal cavity, the method comprising:

Reducing the volume of sample held in the sample container to a volume less than said original volume.

2. The method of claim 1, wherein reducing said volume comprises providing an alternative sample container having an internal cavity having a volume less than said original volume.

3. The method of claim 1, wherein reducing said volume comprises placing an insert into an internal cavity of a sample container.

4. The method of claim 3, wherein said insert comprises an insert cavity configured to hold said sample, wherein said insert cavity comprises a volume less than said original volume.

5. The method of claim 3, wherein said insert is configured effective that optical signals indicative of the presence of, or quantification of, a target substance in a sample contained within said insert may be detected by an optical detector.

6. A device for reducing the volume of sample held within a sample container, wherein said sample container is configured to hold a first volume of sample effective to allow detection of a target substance in said sample by a detector disposed externally to said sample container, wherein said device comprises an insert configured to i) fit within said sample container, ii) hold a second volume of sample, wherein said second volume of sample is less than said first volume of sample, and iii) allow the detection of a signal indicative of the presence of, or quantification of, a target substance in a sample contained within said insert, wherein said detection comprises detection by a detector disposed externally to said sample container.

7. The device of claim 6, wherein said insert is configured to allow the passage of light effective that optical signals indicative of the presence of, or quantification of, a target

substance in a sample contained within said insert may be detected by an optical detector disposed externally to the sample container.

8. A kit for reducing the volume of sample required for performance of an assay, comprising:

An insert configured to fit within a sample container, where said sample container is configured to hold a first volume of sample, and said insert is configured to hold a second volume of sample, where said second volume of sample is a smaller volume than said first volume of sample; and

Instructions for the use of said insert.

9. A system for performance of an assay on a sample, comprising:

An analysis device;

A sample container configured to hold a first volume of sample and for use with said analysis device; and

An insert configured to fit within said sample container, and to hold a second volume of sample, where said second volume of sample is a smaller volume than said first volume of sample.

10. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the intensity of illumination applied to said sample, as compared to the original intensity of illumination applied to the sample, wherein said illumination is used to detect the presence of said target substance in the sample, or to quantify the amount of said target substance in the sample.

11. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said

assay, wherein said assay comprises the detection of a fluorescent label for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the intensity of light illuminating the sample and assay reagents during fluorescence measurements, as compared to the original intensity of light illuminating the sample and assay reagents during fluorescence measurements, wherein said fluorescence measurements are used to detect the presence of said target substance in the sample, or to quantify the amount of said target substance in the sample.

12. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a dye for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the concentration of dye added to the sample, as compared to the original concentration of dye added to the sample, wherein said dye labels a target substance in the sample.

13. The method of claim 12, wherein increasing said concentration of a dye added to the sample comprises increasing the amount of dye added to the sample, as compared to the original amount of dye added to the sample.

14. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an enzymatic label for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the concentration of substrate added to the sample in the presence of the enzyme prior to, or during, enzymatic label measurements, as compared to the original concentration of substrate added to the sample, wherein said enzymatic label measurements are indicative of the presence of, or quantities of, target substance in the sample.

15. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a labeled antibody for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the concentration of labeled antibody added to the sample, as compared to the original concentration of labeled antibody added to the sample, wherein said antibody binds a target substance in the sample.

16. The method of claim 15, wherein increasing said concentration of a labeled antibody added to the sample comprises increasing the amount of labeled antibody added to the sample, as compared to the original amount of labeled antibody added to the sample.

17. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a labeled antibody for detection of the presence of, or quantification of, a target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the number of labels per labeled antibody added to the sample, as compared to the original number of labels per labeled antibody added to the sample, wherein said antibody binds a target substance in the sample.

18. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the sensitivity of an optical detector used to detect said optical signal, as compared to the original sensitivity of the optical detector used to detect the optical signal.

19. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Decreasing the separation between the sample and an optical detector used to detect said optical signal, as compared to the original separation between the sample and the optical detector used to detect the optical signal.

20. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said

assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the path length within the sample between a source of illumination and through the sample to an optical detector used to detect said optical signal, as compared to the original path length within the sample between a source of illumination and through the sample to an optical detector used to detect the optical signal.

21. The method of claim 20, wherein said path length within the sample is increased, as compared to the original path length within the sample, by alteration of the container holding the sample.

22. The method of claim 20, wherein said path length within the sample is increased, as compared to the original path length within the sample, by reflection or refraction of light within the container holding the sample.

23. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Altering the wavelength of light passing through said sample and to an optical detector used to detect the optical signal, as compared to the original wavelength of light passing through the sample and to an optical detector used to detect the optical signal.

24. The method of claim 23, wherein said altering the wavelength of light passing through said sample and to an optical detector used to detect the optical signal comprises providing multiple wavelengths of light passing through the sample and to an optical detector.

25. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an optical signal produced by a target substance, or by a reagent which binds to or reacts with said target substance, the assay being useful for detection of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Altering the polarization of light passing through said sample and to an optical detector used to detect the optical signal, as compared to the original polarization of light passing through the sample and to an optical detector used to detect the optical signal.

26. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of an electrical signal indicative of the presence of, or quantification of, the target substance in a sample, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Increasing the electronic amplification of said electrical signal, as compared to the original electronic amplification of the electrical signal.

27. The method of claim 26, wherein said electrical signal is produced by the target substance, or by a reagent which binds to or reacts with said target substance.

28. A method for reducing the volume of sample used in the performance of an assay as compared to an original volume of sample used for the original performance of said assay, wherein said assay comprises the detection of a signal indicative of the presence of, or quantification of, the target substance in a sample, wherein said signal comprise a temperature-sensitive signal, the method comprising:

Reducing the volume of sample used in the assay from a first sample volume to a second sample volume, wherein said second sample volume is less than said first sample volume, and wherein the assay was originally performed using said first volume of sample; and

Altering the temperature of the assay.

29. The method of claim 28, wherein said temperature-sensitive signal is increased with increasing temperature, and wherein altering the temperature of the assay comprises increasing the temperature of the assay.

30. The method of claim 28, wherein the temperature of said original performance of said assay was near 20 °C, and wherein said altered assay temperature is selected from about 25 °C, about 30 °C, about 32 °C, about 34 °C, about 35 °C, about 36 °C, about 37 °C, about 38 °C, about 39 °C, and about 40 °C.

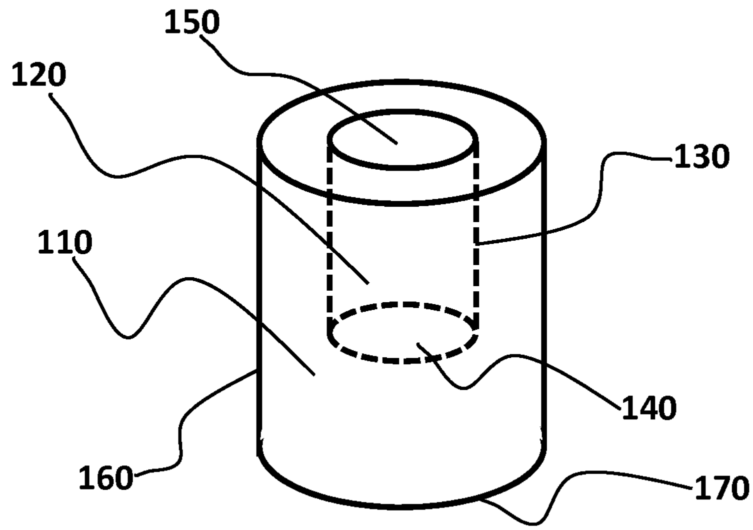

Fig. 1

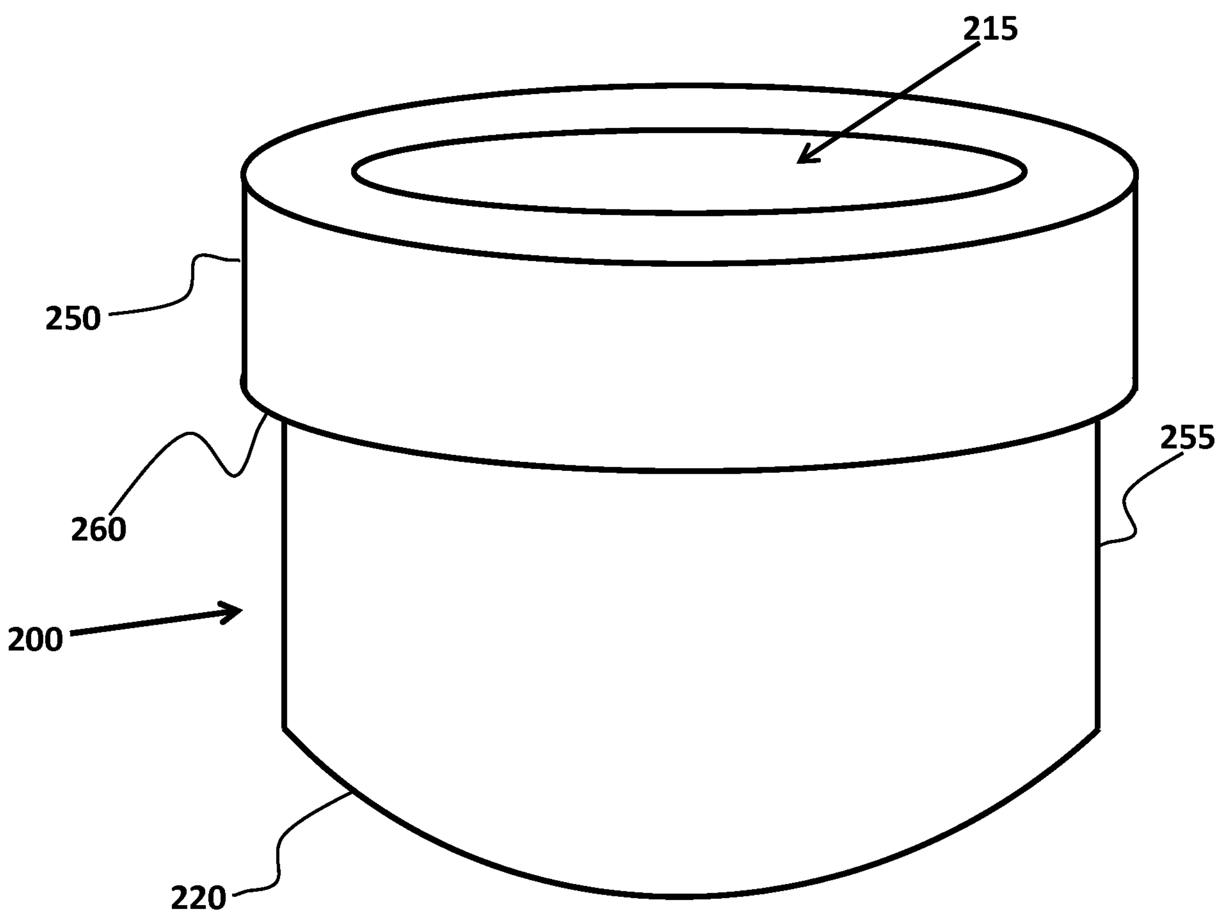

Fig. 2A

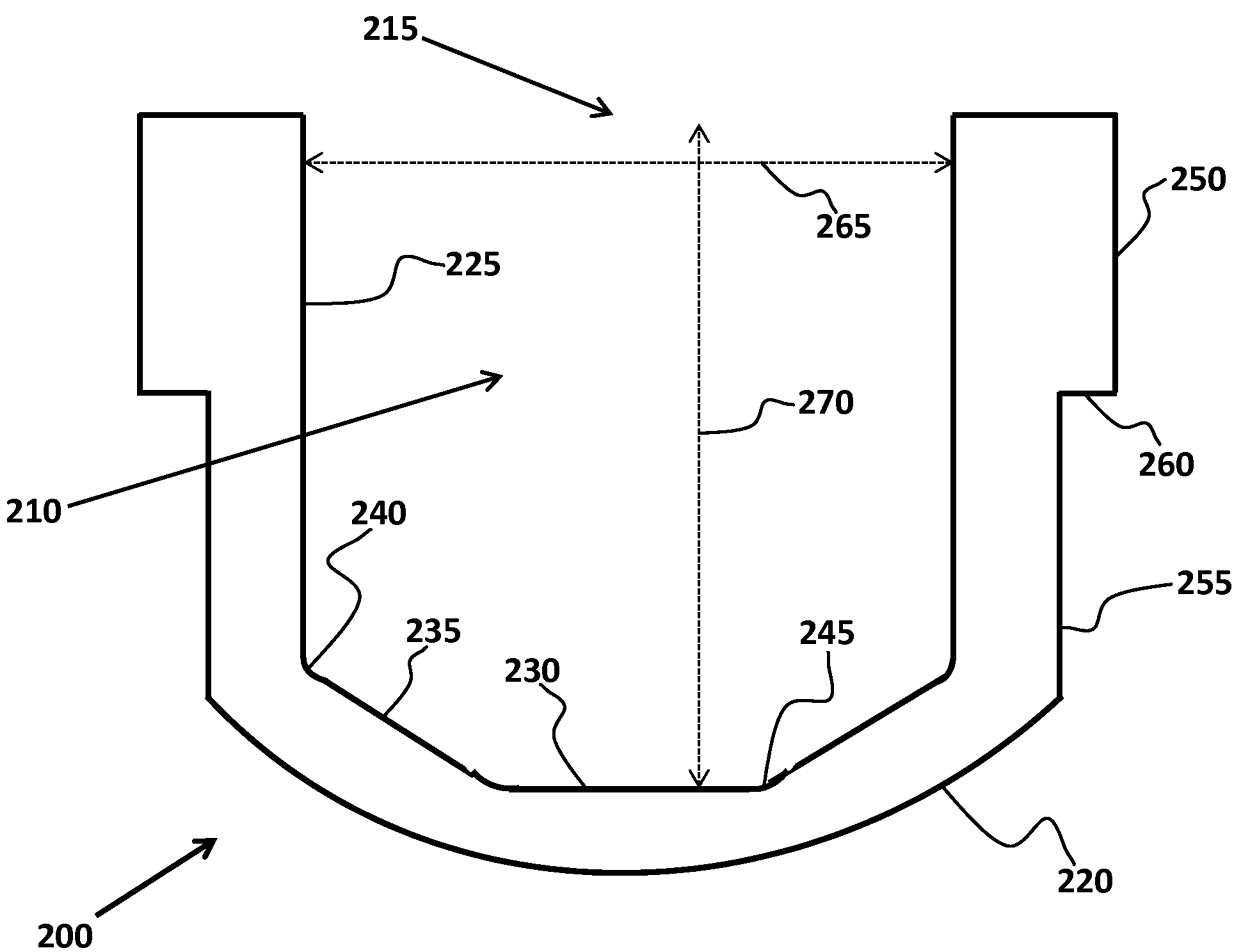

Fig. 2B

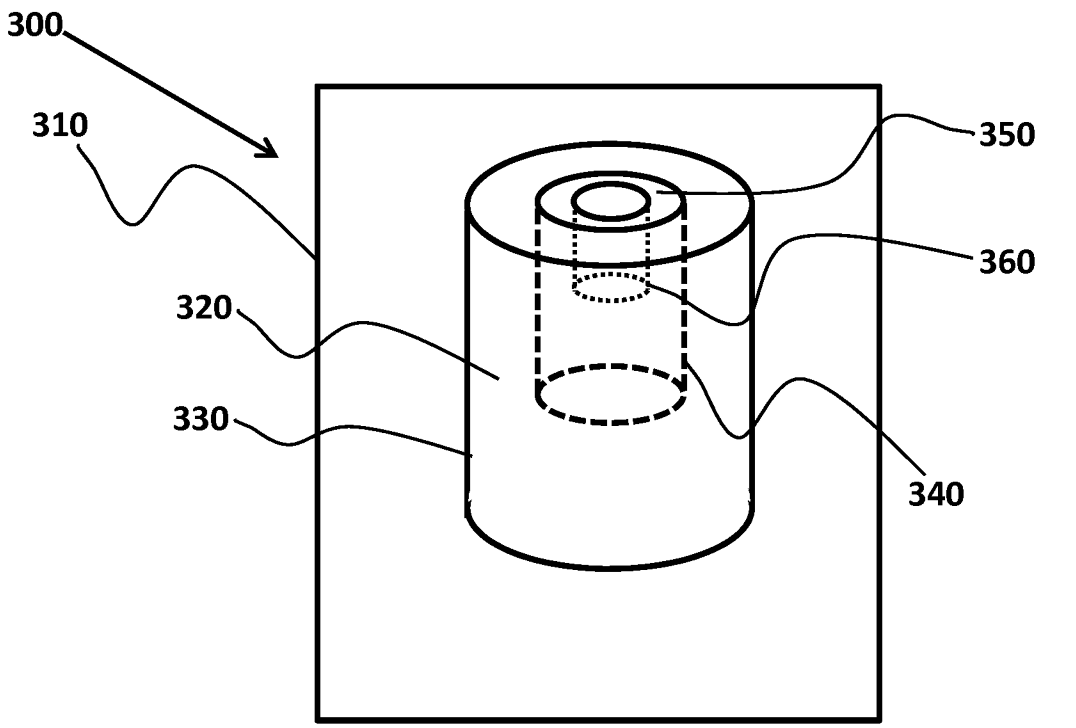

Fig. 3A

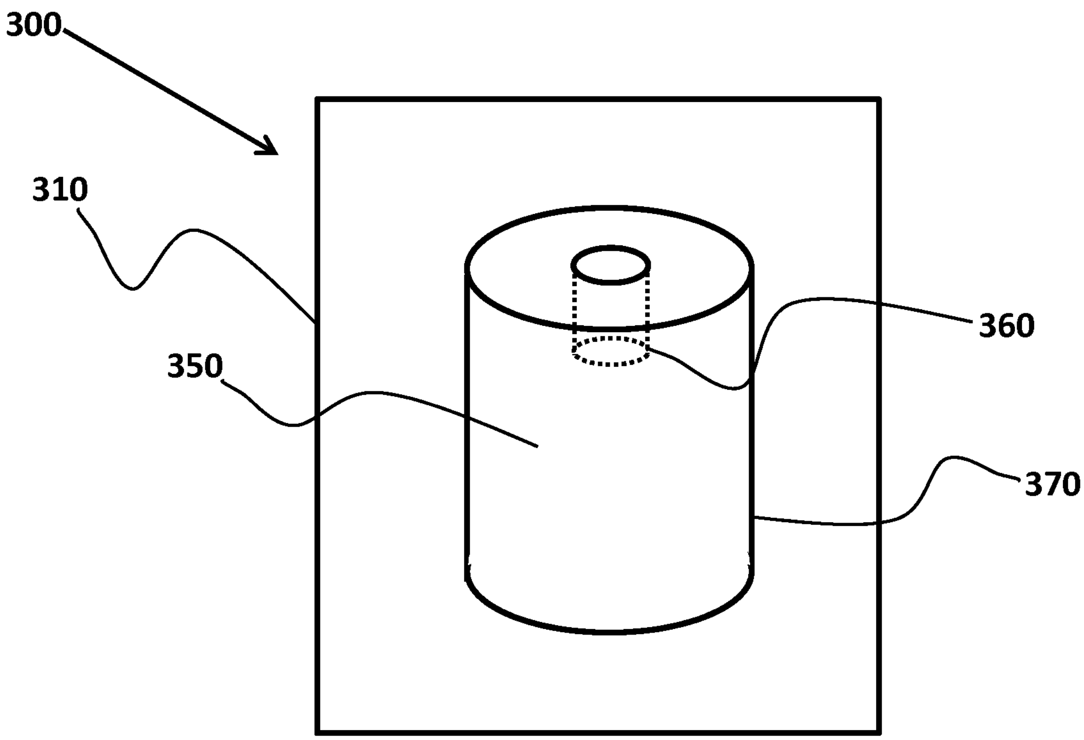

Fig. 3B

Electronic Acknowledgement Receipt

EFS ID:	16805722
Application Number:	61875678
International Application Number:	
Confirmation Number:	5007
Title of Invention:	DEVICES, METHODS AND SYSTEMS FOR REDUCING SAMPLE VOLUME
First Named Inventor/Applicant Name:	. .
Customer Number:	107075
Filer:	James Fox
Filer Authorized By:	
Attorney Docket Number:	3013.101
Receipt Date:	09-SEP-2013
Filing Date:	
Time Stamp:	23:35:42
Application Type:	Provisional

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		3013_101_APP_Reducing_Sample_Volume.pdf	348085 34fd7cc806b236451a50c055557ca45cba33a29c	yes	51

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	39
	Claims	40	47
	Abstract	48	48
	Drawings-only black and white line drawings	49	51
Warnings:			
Information:			
	Total Files Size (in bytes):	348085	

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

EXHIBIT 10

Message

From: Fife, Elizabeth [Elizabeth.Fife@fda.hhs.gov]
Sent: 7/7/2015 6:23:47 PM
To: Brad Arington [/O=THERANOS ORGANIZATION/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=Brad Arington22b]
CC: Lovell, Stephen [Stephen.Lovell@fda.hhs.gov]; El Mubarak, Haja Sittana [HajaSittana.ElMubarak@fda.hhs.gov]
Subject: Corrected/Resent SE Documents - K143236
Attachments: K143236.510kSummary.FINAL_Corrected_20150706.pdf; K143236.IFU.FINAL_Corrected_20150706.pdf; K143236.Letter.SE.FINAL_Corrected_20150707.pdf

Dear Mr. Arrington,

Please see the attached SE Package documents. The corrected document(s) are indicated by "corrected" in the file name.

Thank you,

Elizabeth (Lisa) Fife

Management Analyst

301-796-0769

Food and Drug Administration | Center for Devices and Radiological Health

Office of In Vitro Diagnostics and Radiological Health | Division of Program Operations and Management

10903 New Hampshire Ave

WO66 RM4500

Silver Spring, MD 20993-0002

510(k) SUMMARY K143236

I. GENERAL INFORMATION

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Associate Director, Regulatory
Phone: 650-856-7304
Fax: 650-838-9165
Email: barington@theranos.com

Date Prepared: June 29, 2015

II. DEVICE INFORMATION

Trade Name:	Theranos™ Herpes Simplex Virus-1 (HSV-1) IgG Assay
Common Name:	HSV-1 IgG assay
Regulation Number:	21 CFR§866.3305
Regulation Name:	Herpes simplex virus serological assays
Regulatory Class:	Class II
Product Code:	MXJ (Enzyme Linked Immunosorbent Assay, Herpes Simplex Virus, HSV-1)
Panel:	Microbiology (83)

III. PREDICATE DEVICE

HerpeSelect® 1 and 2 Immunoblot IgG (K000238; Focus Diagnostics, Inc.)

IV. DEVICE DESCRIPTION

The Theranos HSV-1 IgG Assay is for use with the Theranos System. The Theranos System performs automated sample processing steps and analysis to produce the test results.

The Theranos HSV-1 IgG Assay is a three-step sandwich immunoassay with an HSV-1 glycoprotein G (gG) recombinant antigen coated surface, an anti-human IgG detection reagent conjugated to alkaline phosphatase (AP) and chemiluminescent substrate. During the first incubation step, the HSV-1 IgG antibodies present in the positive control and sample bind to the gG recombinant antigen on the coated surface. Following the first incubation step, unbound materials are removed with a wash cycle. Then the detection reagent-AP conjugate is added and during the second incubation step, the detection reagent-AP conjugate reacts with the HSV-1 IgG antibodies already bound to the capture surface. Following the second incubation, unbound materials are removed with a wash cycle. The chemiluminescent substrate is added to the capture-analyte-detection complex during the third incubation step to initiate the chemiluminescence reaction. Light generated by this reaction is detected and analyzed by the Theranos System using a calibration function to determine the cut-off index (COI) values for the sample and controls. The results for the Positive and Negative controls must be within specified limits for a run to be considered valid.

V. INDICATIONS FOR USE

The Theranos™ HSV-1 IgG Assay is a chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to herpes simplex virus type 1 (HSV-l) in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-l infection. The predictive value of positive and negative results depends on the population's prevalence and the pretest likelihood of HSV-1.

The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients.

The Theranos HSV-1 IgG Assay is for use with the Theranos System which performs automated sample processing steps and result analysis.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

Table 1: Similarities between the Theranos HSV-1 IgG Assay and the Predicate

Characteristic	Theranos™ HSV-1 IgG Assay **(K143236)**	Focus HerpeSelect® 1 and 2 Immunoblot IgG **(K000238)**
Intended use	Qualitative test to detect presence or absence of IgG antibodies to HSV-1 in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. Indicated for testing sexually active individuals and expectant mothers as an aid in presumptive diagnosis of HSV-1 infection. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 infection.	Qualitative test to detect presence or absence of IgG antibodies to HSV-1 and HSV-2 in human sera. Indicated for testing sexually active adults or expectant mothers as an aid in presumptive diagnosis of HSV-1 and HSV-2 infection. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2 infection.

Table 2: Differences between the Theranos HSV-1 IgG Assay and the Predicate

Characteristic	Theranos™ HSV-1 IgG Assay **(K143236)**	Focus HerpeSelect® 1 and 2 Immunoblot IgG **(K000238)**
Specimen Types	• Venous serum, • K2-EDTA anticoagulated human plasma from venous blood, • Human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes	• Venous Serum
Type of Assay	Chemiluminescent immunoassay	Nitrocellulose Immunoblot
Sample Handling	Automated sample handling/processing	Manual sample handling/processing
Capture Reagent	HSV-1 recombinant antigen (gG1)	HSV-1/HSV-2 antigen immobilized on nitrocellulose membrane

VII. PERFORMANCE

To characterize performance of the Theranos HSV-1 IgG immunoassay the following studies were conducted:

<u>Precision – CLIA Laboratory Model, Venous Serum</u>

A study for estimating the precision of the Theranos HSV-1 IgG Assay for venous serum samples in a CLIA Laboratory model was performed by testing a panel of 6 serum samples spanning the analytical range [negative (A), high negative (B), equivocal (C), low positive (D), moderate positive (E), and positive (F)]. The precision study was conducted at one site with thirty five (35) TSPU devices, three (3) lots of cartridges and sixteen (16) operators in total. The study duration was 13 days in total. Details of the study design for different samples are presented in Table 3 below.

Table 3: Design of Precision Study: Numbers of Replicates, Devices, Days and Operators

Panel Member	Valid Replicates				No. of Devices	No. of Days	No. of Operators	No. of Invalid Replicates
	Total	Lot 1	Lot 2	Lot 3				
A (Neg.)	91	26	38	27	35	7	14	3
B (High Neg.)	88	24	37	27	28	7	14	2
C (Equivocal)	78	27	44	8*	35	8	16	3
D (Low Pos.)	80	25	27	28	11	2	4	4
E (Mod. Pos.)	64	25	13	26	13	2	6	1
F (Pos.)	69	25	19	25	15	2	4	3

*Sufficient cartridges from reagent lot #3 were not available.

Results of the precision study are presented in Table 4.

Table 4: Summary of Precision Study Results

Panel Member	Mean (COI)		Repeatability (same device, same lot)	Between-device	Between-lot	Precision (same device, different lot)	Precision (different device, same lot)	Precision (different device, different lot)
A (Neg.)	0.425	SD	0.049	0.007	0.000	0.049	0.049	0.049
		%CV	11.5%	1.6%	0%	11.5%	11.6%	**11.6%**
B High Neg.)	0.648	SD	0.086	0.011	0.029	0.091	0.087	0.092
		%CV	13.3%	1.7%	4.5%	14.1%	13.4%	**14.2%**
C Equivocal)	1.016	SD	0.093	0.062	0.065	0.113	0.112	0.129
		%CV	9.1%	6.1%	6.4%	11.1%	11.0%	**12.7%**
D (Low Pos.)	1.727	SD	0.208	0.098	0.013	0.208	0.230	0.230
		%CV	12.0%	5.7%	0.8%	12.0%	13.3%	**13.3%**
E (Mod. Pos.)	3.809	SD	0.305	0.276	0.108	0.324	0.411	0.425
		%CV	8.0%	7.3%	2.8%	8.5%	10.8%	**11.2%**
F (Pos.)	8.996	SD	0.807	0.437	0.000	0.807	0.918	0.918
		%CV	9.0%	4.9%	0.0%	9.0%	10.2%	**10.2%**

Table 5 presents percent of invalid results and percents of negative, equivocal and positive among valid results for each sample.

Table 5: Percent of Invalid Results and Percents of Negative, Equivocal and Positive among Valid Results

Panel Member	Mean (COI)	Number of Replicates	Percent of Invalid	Percent of Negative among Valid	Percent of Equivocal among Valid	Percent of Positive among Valid
A (Neg.)	0.425	94	3.2% (3/94)	100% (91/91)		
B (High Neg.)	0.648	90	2.2% (2/90)	100% (88/88)		
C (Equivocal)	1.016	81	3.7% (3/81)	17.9% (14/78)	60.3% (47/78)	21.8% (17/78)
D (Low Pos.)	1.727	84	4.8% (4/84)			100% (80/80)
E (Mod. Pos.)	3.809	65	1.5% (1/65)			100% (64/64)
F (Pos.)	8.996	72	4.2% (3/72)			100% (69/69)

The results of the study demonstrate that the precision of the Theranos HSV-1 IgG Assay (including different TSPU devices, different lots of cartridges, and different operators) when performed in a CLIA Laboratory was in the range 10.2% to 14.2%.

<u>Precision – CLIA Laboratory Model, Fingerstick Whole Blood</u>

A study for estimating the precision of the Theranos HSV-1 IgG Assay for fingerstick whole blood samples in a CLIA Laboratory model was performed by testing a panel of 3 fingerstick plasma samples spanning the analytical range [high negative (P), equivocal (Q), moderate positive (R)]. The precision study was conducted at one site with thirty six (36) TSPU devices, three (3) lots of cartridges and nine (9) operators in total. The study duration was 4 days in total. Details of the study design for different samples are presented in Table 6 below.

Table 6: Design of Precision Study: Numbers of Replicates, Devices, Days and Operators

Panel Member	Valid Replicates				No. of Devices	No. of Days	No. of Operators	No. of Invalid Replicates
	Total	Lot 1	Lot 2	Lot 3				
P (High Neg.)	168	56	56	56	30	4	9	3*
Q (Equivocal)	168	56	56	56	29	4	9	2*
R (Mod. Pos.)	168	56	56	56	27	4	9	2*

*All invalid replicates were repeated

Results of the precision study are presented in Table 7.

Table 7: Summary of Precision Study Results

Panel Member	Mean (COI)		Repeatability (same device, same lot)	Between-device	Between-lot	Precision (same device, different lot)	Precision (different device, same lot)	Precision (different device, different lot)
P (High Neg.)	0.888	SD	0.083	0.006	0.050	0.096	0.083	0.097
		%CV	9.3%	0.7%	5.6%	10.9%	9.3%	**10.9%**
Q (Equivocal)	1.047	SD	0.094	0.025	0.069	0.117	0.098	0.119
		%CV	9.0%	2.4%	6.6%	11.1%	9.3%	**11.4%**
R (Mod. Pos.)	3.241	SD	0.342	0.122	0.157	0.377	0.363	0.396
		%CV	10.6%	3.8%	4.9%	11.6%	11.2%	**12.2%**

Table 8 presents percent of invalid results and percents of negative, equivocal and positive among valid results for each sample.

Table 8: Percents of Positive, Equivocal, Negative and Invalid Results

Panel Member	Mean (COI)	Number of Replicates	Percent of Invalid	Percent of Negative among Valid	Percent of Equivocal among Valid	Percent of Positive among Valid
P (High Neg.)	0.888	171	1.8% (3/171)	58.3% (98/168)	40.5% (68/168)	1.2% (2/168)
Q (Equivocal)	1.047	170	1.2% (2/170)	6.5% (11/168)	63.1% (106/168)	30.4% (51/168)
R (Mod. Pos.)	1.016	170	1.2% (2/170)			100% (168/168)

The results of the study demonstrate that precision of the Theranos HSV-1 IgG Assay (including different TSPU devices, different lots of cartridges, and different operators) when performed in a CLIA Laboratory was in the range from 10.9% to 12.2%.

Reproducibility

A study designed to process multiple fingerstick whole blood samples from individual subjects was performed to evaluate the reproducibility of the Theranos HSV-1 IgG Assay when used with Theranos Capillary Tubes and Nanotainer Tubes. The study was conducted at 3 collection sites with 10 subjects at each site. From each of 30 subjects, 9 Capillary Tubes and Nanotainer Tubes from 3 manufacturing lots (i.e. 3 Capillary Tubes and Nanotainer Tubes per lot) and 2 serum separator tubes (SSTs) were collected. Each subject had the following measurements:

- Each of the 9 Capillary Tubes and Nanotainer Tubes was tested. These data were used for evaluation of between-Capillary Tubes and Nanotainer Tubes imprecision, between-lot imprecision and total imprecision that includes between-Capillary Tubes and Nanotainer Tubes and between-lot imprecisions.
- One Nanotainer Tube (from one of the 3rd lot of Capillary Tubes and Nanotainer Tubes for each subject) was tested in duplicate via recovering a sample from one

Capillary Tubes and Nanotainer Tubes and transferring a sample to another Capillary Tubes and Nanotainer Tubes. These data were used for evaluation of within-Capillary Tubes and Nanotainer Tubes imprecision.

- Each of the 2 SSTs was tested. These data were used for evaluation of between-SST imprecision.

For samples with mean COI value at the baseline ≥0.5, percent differences were calculated and for samples with mean COI value at the baseline <0.5, differences were calculated. Table 9 summarizes the results of the reproducibility study broken down by collection site and by high or low COI subjects; the variability metrics are averaged across all subjects within the site.

Table 9: Summary of Results of the Reproducibility Study

Collection Site	Subjects	Capillary Tubes and Nanotainer Tubes				SST
		Within-Capillary Tubes and Nanotainer Tubes (%CV or SD)	Between-Capillary Tubes and Nanotainer Tubes (%CV or SD)	Between-Lot (%CV or SD)	Total (%CV or SD)	Between-SST (%CV or SD)
1	6 subjects with COI values 1.4 – 13.5	%CV=6.0%	%CV = 9.0%	%CV = 6.8%	%CV=12.6%	%CV=9.6%
	4 subjects with COI values 0.03-0.28	SD=0.008	SD = 0.015	SD = 0.016	SD = 0.024	SD=0.11
2	7 subjects with COI values 1.6 – 16.8	%CV=8.2%	%CV=9.2%	%CV=3.2%	%CV=10.8%	%CV=12.5%
	3 subjects with COI values 0.07-0.19	SD=0.009	SD=0.011	SD=0.008	SD=0.015	SD=0.019
3	5 subjects with COI values 4.5 – 14.3	%CV=8.2%	%CV=8.1%	%CV=6.0%	%CV=11%	%CV=12.4%
	5 subjects with COI values 0.02-0.32	SD=0.08	SD=0.019	SD=0.013	SD=0.025	SD=0.021
Combined	18 subjects with COI values 1.4-16.8	%CV=7.5%	%CV=8.8%	%CV=5.2%	%CV=11.4%	%CV=11.5%
	12 subjects with COI values 0.02-0.32	SD=0.008	SD=0.015	SD=0.013	SD=0.022	SD=0.017

- Within-Capillary Tubes and Nanotainer Tubes imprecision was %CV=7.5% for aggregated subjects with mean COI ≥ 0.5 and SD=0.008 for aggregated subjects with a mean COI < 0.5.

- Total imprecision including between-Capillary Tubes and Nanotainer Tubes and between-lot imprecisions was %CV= 11.4% for aggregated subjects with a mean COI ≥ 0.5 and SD=0.022 for aggregated subjects with a mean COI < 0.5.

- Between-serum separator tubes imprecision was %CV=11.5% for aggregated subjects with a mean COI ≥ 0.5 and SD=0.017 for aggregated subjects with a mean COI < 0.5.

Analyte Stability

An analyte stability study was performed to characterize the stability of HSV-1 IgG in clinical matrices as measured by the Theranos HSV-1 IgG Assay under different sample storage conditions and time periods. A summary of analyte storage conditions and durations for different sample types and matrices claimed for the Theranos HSV-1 IgG Assay is presented in Table 10.

Table 10: Summary of Analyte Storage Conditions and Durations

Condition	Venous Serum	Venous K2-EDTA Plasma	Fingerstick K2-EDTA Plasma	Fingerstick K2-EDTA Whole Blood
Stored at 2-8°C	48 hr	48 hr	48 hr	48 hr
Stored at room temperature (20-25°C)	6 hr	6 hr	6 hr	6 hr
Stored at -20°C	1 week	1 week	1 week	N/A
Freeze/thaw cycles	3	3	3	N/A

Within 2 hours after collection, one aliquot of each sample type or matrix was tested with the Theranos HSV-1 IgG Assay in duplicate, to establish the value at baseline. The samples were stored in Nanotainer Tubes under the appropriate conditions. Comparison of an average of two replicates at the predetermined time points with the average of two replicates at baseline was performed. For samples with a mean COI value at the baseline ≥0.5, percent differences were calculated and for samples with a mean COI value at the baseline <0.5, differences were calculated.

Acceptance criteria were as follows: i) a difference averaged over all samples with baseline mean COI value ≥0.5 must be less than ±10% and a difference averaged over all samples with baseline COI mean <0.5 must be less than 0.02 and ii) for each sample, an observed difference must be less than 15% for the samples with baseline mean COI value ≥0.5 and must be less than 0.08 for the samples with baseline mean COI value <0.5 (the range of differences expected if there is no effect of storage on the HSV-1 IgG analyte). The results are summarized in Table 11.

Table 11: Summary of Mean Absolute Difference Measures for all Storage Conditions and Sample Types or Matrices

	Sample Type and Matrix	Samples with a Baseline COI < 0.5		Samples with a Baseline COI > 0.5	
		Difference averaged over all samples	The largest observed difference among samples	Percent difference averaged over all samples	The largest observed percent difference among samples
Stored at 2-8C, 48 hrs	Venous serum	0.006	0.006	1.0%	13.6%
	Venous K2-EDTA plasma	-0.007	-0.007	2.3%	13.3%
	Fingerstick K2-EDTA plasma from whole blood	0.003	0.003	-0.4%	13.9%
Stored at -20, 1 week	Venous serum	0.008	0.015	0.8%	13.3%
	Venous K2-EDTA plasma	0.003	0.005	-1.0%	12.7%
	Fingerstick K2-EDTA plasma from whole blood	0.001	0.008	1.8%	-13.9%
Freeze thaw cycles, n=3	Venous serum	0.007	0.021	-0.1%	13.6%
	Venous K2-EDTA plasma	0.021	0.037	-1.7%	-13.4%
	Fingerstick K2-EDTA plasma from whole blood	0.006	0.022	-1.0%7	13.6%
Stored at room temp, 6 hrs	Venous serum	-0.001	-0.011	-3.2%	-11.9%
	Venous K2-EDTA plasma	0.002	0.022	0.1%	13.7%
	Fingerstick K2-EDTA plasma from whole blood	-0.004	-0.026	1.1%	13.9%

Interfering Substances

A study was designed and performed (in accordance with CLSI EP07-A2) to evaluate the performance of the Theranos HSV-1 IgG Assay in the presence of potentially interfering substances to assess the impact of these endogenous substances and commonly used drugs on the performance of the Theranos HSV-1 IgG Assay. Interferents were tested with three serum samples (negative (mean COI 0.024), high negative (mean COI 0.77) and low positive (mean COI 1.52)) that were contrived by using a high positive sample and diluting it with pooled negative serum. Samples were spiked with the interferent at levels shown in Table 12. Each serum pool was tested in duplicate.

For the low positive and the high negative pools, the acceptance criteria were a mean recovery within +/- 20% of the value of the unspiked sample (i.e., in the absence of the potential interferent or drug). All low positive and high negative samples showed a signal change of less than 15% for all interfering substances. All positive samples remained positive and all negative samples remained negative upon spiking of drug or other interferents. For the negative pool, the acceptance criterion was a deviation of less than 0.02 COI. All negative samples showed a mean deviation of ≤0.02 COI, except Intralipid. Intralipid spikes did not show any effect on recovery for near cut-off samples, high negative and low positive samples.

Table 12: Summary of Interfering Substances Studies: Endogenous Interferents and Drug Interferents

		Negative Pool		High Negative Pool		Low Positive Pool	
Interferent	Level	Mean COI	ΔCOI	Mean COI	% Recovery	Mean COI	% Recovery
Hemoglobin	1000 mg/dL	0.025	0.00	0.69	90	1.71	113
Bilirubin	20 mg/dL	0.024	0.00	0.68	88	1.61	106
Intralipid	2000 mg/dL	0.053	0.03	0.81	105	1.60	105
Acetylcysteine	150 mg/L	0.019	-0.004	0.68	88	1.40	92
Ampicillin-Na	1000 mg/L	0.025	0.001	0.76	99	1.44	95
Ascorbic acid	300 mg/L	0.027	0.003	0.75	97	1.67	110
Ca-Dobesilate	200 mg/L	0.027	0.004	0.70	91	1.51	99
Cyclosporine	5 mg/L	0.031	0.008	0.74	97	1.53	101
Cefoxitin	2500 mg/L	0.027	0.003	0.74	97	1.52	100
Heparin	5000U	0.020	-0.003	0.80	103	1.52	100

		Negative Pool		High Negative Pool		Low Positive Pool	
Interferent	Level	Mean COI	ΔCOI	Mean COI	% Recovery	Mean COI	% Recovery
Levodopa	20 mg/L	0.030	0.006	0.68	88	1.42	94
Methyldopa+1.5h20	20 mg/L	0.024	0.000	0.74	97	1.37	90
Metronidazole	200 mg/L	0.039	0.016	0.74	96	1.38	91
Phenylbutazone	400 mg/L	0.021	-0.002	0.74	96	1.42	94
Doxycycline	50 mg/L	0.024	0.000	0.71	92	1.35	89
Acetylsalicylic acid	1000 mg/L	0.026	0.002	0.75	97	1.37	90
Rifampicin	60 mg/L	0.014	-0.009	0.69	90	1.35	89
Acetaminophen	200 mg/L	0.034	0.010	0.64	83	1.68	111
Control		0.024	0.000	0.77	100	1.52	100

Cross-reactivity

A study was performed to evaluate the performance of the Theranos HSV-1 IgG Assay in the presence of IgG antibodies against twenty-one (21) infectious agents defined as potential cross-reactants in the FDA guidance on HSV serological assays. Banked serum samples confirmed positive for IgG against the infectious agents of interest were acquired from commercial vendors. At least three (3) samples, independently confirmed as positive for that agent and negative for HSV-1 IgG on the reference method, were tested on the Theranos HSV-1 IgG Assay in order to rule out cross-reactivity of the Theranos HSV-1 IgG Assay with IgG against a potential cross reactant. The results of this study are displayed in Table 13.

Table 13: Summary of Cross-reactivity Study on Theranos HSV-1 IgG Assay

Organism/Condition	No.	Reference HSV-1 Assay	Theranos HSV-1 Positive	Theranos HSV-1 Negative	Theranos HSV-1 Equivocal
Epstein Barr Virus (IgG)	6	Negative	0	6	0
Epstein Barr Virus (IgM)	1	Negative	0	1	0
HPV	4	Negative	0	4	0
Rubella (IgG)	13	Negative	0	13	0
HSV-2 (IgG)	40	Negative	0	40	0
HAMA samples	4	Negative	0	4	0
Treponema pallidum	8	Negative	0	7	1*
Rheumatoid Factor (RF)	8	Negative	1**	7	0
Anti-nuclear antibody (ANA)	8	Negative	0	8	0
Sjogren's Syndrome	3	Negative	0	3	0
CMV (IgG)	5	Negative	0	5	0
CMV (IgM)	2	Negative	0	2	0
Chlamydia trachomatis (IgG)	10	Negative	0	10	0
HCV (IgG)	3	Negative	0	3	0
HBsAg	3	Negative	0	3	0
VZV IgG	5	Negative	0	5	0
Measles IgG	5	Negative	0	5	0
HIV-1 (IgG)	4	Negative	0	4	0
Toxoplasma IgG	4	Negative	0	4	0
Candida albicans Ag	3	Negative	0	3	0
Systemic Lupus	3	Negative	0	3	0

*Systematic cross-reactivity ruled out (7/8 samples in same category tested negative)
**Confirmed as positive upon retest by Theranos HSV-1 assay; systematic cross-reactivity ruled out (7/8 samples in same category tested negative)

Assay Cut-off

A study was performed to establish the cut-off and the limits of the equivocal zone for the Theranos HSV-1 IgG Assay using 192 serum samples. Then 120 independent serum samples were analyzed to validate the established cut-off. The calibrators were assigned COI values based on the established assay cut-off, the cut-off for positive results a COI of 1.1 and cut-off for negative results a COI of 0.9. The results of the cut-off validation study are displayed in Table 14 below.

Table 14: Performance of Selected Cut-off on Independent Sample Set

Agreement Classification	Percent Agreement	95% Confidence Interval
NPA	96.0% (47/49)	86.3-98.9
PPA	97.1% (69/71)	90.3-99.2

Fingerstick Plasma – CLIA Laboratory Model

To demonstrate the performance of the Theranos HSV-1 IgG Assay for fingerstick whole blood samples collected at 3 Theranos Patient Service Centers (TPSCs) and processed at the CLIA-certified laboratory.

At each site, fingerstick whole blood samples were collected into a pair of Theranos Capillary Tubes and Nanotainer Tubes, and venous samples were collected into serum tubes from each of 20, 16 and 25 adult subjects at three collection sites.

Samples were shipped refrigerated to the Theranos CLIA-certified laboratory in Palo Alto, CA. Upon receipt, fingerstick whole blood samples in the Nanotainer Tubes were centrifuged at 1200g for 5 minutes. Plasma was extracted and processed and analyzed on the Theranos System. All samples were processed or frozen as plasma within 48 hours of draw.

A summary of the performance information is shown in Table 15.

Table 15: Summary of Method Comparison for Samples Collected at 3 Theranos Patient Service Centers

		Reference Result	
		POS	NEG
Theranos Result	POS	38	0
	NEG	1	22

	Point Estimate	95% Confidence Interval
Sensitivity	97.4% (38/39)	86.8 – 99.6
Specificity	100% (22/22)	85.1 – 100

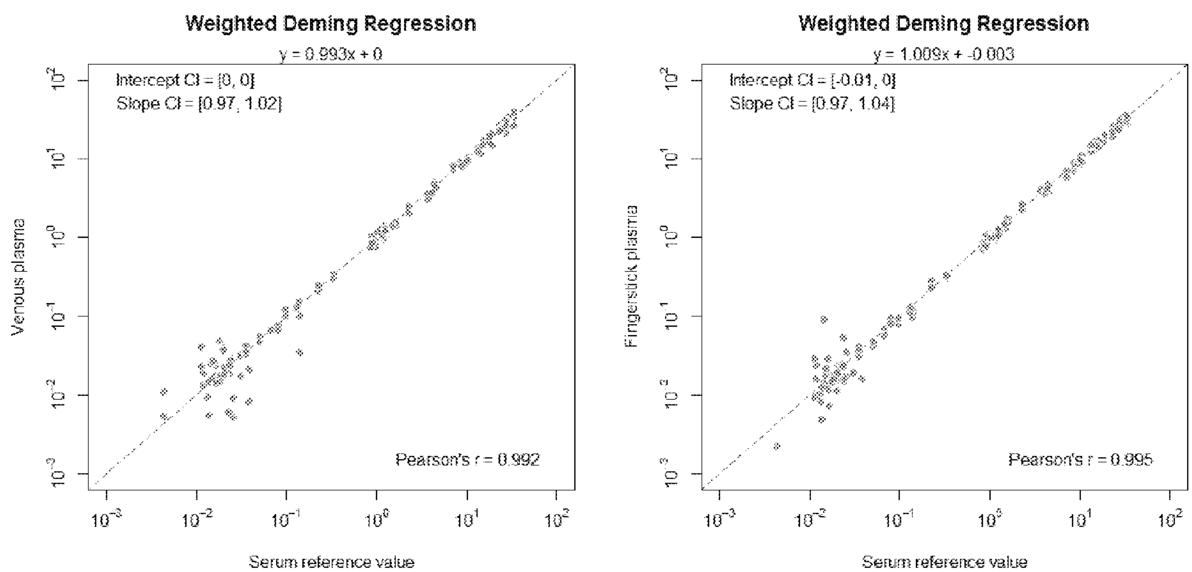
Matrix Comparison

The effect of anticoagulants and different sample types (fingerstick and venous) on the performance of the Theranos HSV-1 IgG Assay was determined by comparing matched venous serum, venous K2-EDTA plasma, and fingerstick K2-EDTA plasma samples from 70 donors. Forty-three matched sample sets were contrived to have analyte values close to the cut-off. The acceptance criterion was a recovery of positive plasma samples within ± 20% of the corresponding serum reference value (serum drawn into primary tubes without gel). For negative samples, the acceptance criteria was a difference of <= 0.02 COI from the corresponding serum value. All anticoagulant–treated plasma samples met this criterion. Weighted Deming regression was performed. The slope and an intercept of the regression line and their 95% confidence intervals along with correlation coefficients are shown in Table 16 and a graphical depiction is shown in Figure 1.

Table 16: Summary of Weighted Deming Regression Analysis Performed on Matrix Equivalency Data for Venous Plasma and Fingerstick Plasma Samples

Sample Type/Matrix	Correlation coefficient	Slope	95% confidence interval on slope	Intercept	95% confidence interval on intercept
Venous plasma	0.992	0.993	[0.967, 1.019]	0.000	[-0.003, 0.003]
Fingerstick plasma	0.995	1.009	[0.973, 1.044]	-0.003	[-0.006, -0.001]

Figure 1: Regression Analysis for Matrix Comparison Study



<u>Expected Values</u>

The Theranos HSV-1 IgG Assay was used to evaluate the prevalence of HSV-1 IgG antibodies in individuals for whom an HSV-1 IgG test was ordered by a physician including pregnant women. The study population for the Theranos HSV-1 IgG Assay consisted of a total of 558 subjects, with 260 sexually active adults and 298 individuals identified as pregnant women. The result for 1 out of the 558 subjects is not reported, as indicated in Table 17 (1 subject), giving a total of 557 subjects. The data for the intended use population (557 specimens) have been summarized according to age group in decades, gender, number of reactive results, number of equivocal results, and number of non-reactive results. The data for the intended use population have been summarized in Table 17 (259 specimens from sexually active adult subjects) and Table 18 (298 specimens from pregnant subjects).

Table 17: Expected Results for Theranos HSV-1 IgG Assay in Sexually Active Adult Subjects

Age Range	Gender	Reactive	Equivocal	Non-Reactive
		N/Total (%)	N/Total (%)	N/Total (%)
16 to 19	Male	0/0 (0)	0/0 (0)	0/0 (0)
16 to 19	Female	1/4 (25)	0/4 (0)	3/4 (75)
20 to 29	Male	8/18 (44.4)	1/18 (5.6)	9/18 (50)
20 to 29	Female	29/73 (39.7)	0/73 (0)	44/73 (60.3)
30 to 39	Male	5/10 (50)	0/10 (0)	5/10 (50)
30 to 39	Female	33/62 (53.2)	0/62 (0)	29/62 (46.8)
40 to 49	Male	5/10 (50)	0/10 (0)	5/10 (50)
40 to 49	Female	16/27 (59.3)	0/27 (0)	11/27 (40.7)
50 to 59	Male	17/20 (85)	0/20 (0)	3/20 (15)
50 to 59	Female	9/11 (81.8)	0/11 (0)	2/11 (18.2)
60 to 69	Male	5/6 (83.3)	0/6 (0)	1/6 (16.7)
60 to 69	Female	5/10 (50)	1/10 (10)	4/10 (40)
70 to 79	Male	3/4 (75)	0/4 (0)	1/4 (25)
70 to 79	Female	1/3 (33.3)	0/3 (0)	2/3 (66.7)
80 to 89	Male	0/0 (0)	0/0 (0)	0/0 (0)
80 to 89	Female	1/1 (100)	0/1 (0)	0/1 (0)
Total*		138/259 (53.3)	2/259 (0.8)	119/259 (45.9)

*1 sample not reported since age information was not available

Table 18: Expected Results for Theranos HSV-1 IgG Assay in Pregnant Subjects

Age Range	Gender	Reactive	Equivocal	Non-Reactive
		N/Total (%)	N/Total (%)	N/Total (%)
18 to 19	Female	13/13 (100)	0/13 (0)	0/13 (0)
20 to 29	Female	114/175 (65.1)	1/175 (0.6)	60/175 (34.3)
30 to 39	Female	61/104 (58.7)	0/104 (0)	43/104 (41.3)
40 to 49	Female	5/6 (83.3)	0/6 (0)	1/6 (16.7)
Total		193/298 (65)	1/298 (0.3)	104/298 (35)

The hypothetical positive and negative predictive values (PPV, NPV) for the two intended use populations are shown in Table 19. The calculations are based on the specificity and sensitivity values for the Theranos HSV-1 IgG Assay determined in the clinical study;

1. Specificity of 97.4% and Sensitivity of 95.1% in sexually active adults
2. Specificity of 95.2% and Sensitivity of 97.4% in pregnant women

Table 19: Hypothetical Predictive Values

Prevalence (%)	Sexually Active Adults		Pregnant Women	
	PPV (%)	NPV (%)	PPV (%)	NPV (%)
50	93.8	92.6	92.1	91.7
45	93.2	93.2	91.3	92.4
40	92.4	93.8	90.3	93.0
35	91.4	94.2	89.1	93.5
30	90.1	94.6	87.5	94.0
25	88.3	94.9	85.3	94.3
20	85.8	95.2	82.3	94.7
15	82.0	95.5	77.7	95.0
10	75.2	95.7	69.9	95.2
5	60.2	96.0	53.8	95.5

Clinical Performance in the Intended Use Populations (CLIA Laboratory Model)

A clinical study was conducted to characterize the performance of the Theranos HSV-1 IgG Assay in the Theranos CLIA-certified Laboratory in comparison to the FOCUS HerpeSelect Immunoblot (as the reference method for performance analysis).

Prospectively collected, archived venous serum samples collected from pregnant women and sexually active adults (18 years and older) who had a prescription for a HSV-1 IgG test. Samples were obtained from multiple specimen sources covering 10 US states and Mexico.

The equivocal results on the Focus HerpeSelect Immunoblot (that repeatedly tested equivocal) were resolved using a validated western blot reference test (University of Washington, Seattle) as per the instructions of the package insert for the reference method. Ten samples from the sexually active adult sub-population tested initially equivocal on the Focus HerpeSelect Immunoblot and were resolved by the University of Washington western blot as 2 negatives and 7 positives. One sample was not resolved. There were no samples in the sexually active adult sub-population that returned an invalid result.

In the pregnant women sub-population, 8 samples tested initially equivocal on the Focus HerpeSelect immunoblot. Of these, 4 samples could not be resolved by the University of Washington western blot due to insufficient volume availability. Of the remaining 4, 1 (one) were resolved as negative and 3 as positive. There were 3 samples that returned an invalid result on the Theranos HSV-1 IgG test. These samples were rerun and resulted in

valid results included in the analysis. The clinical performance results are summarized in Tables 20 and 21.

Table 20: Summary of Theranos HSV-1 IgG Assay Performance in Sexually Active Adult Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	137	0	2	139
	Equivocal	1	0	1	2
	Negative	5	1	113	119
	Total	143	1	116	***260***

	Point Estimate	95% Confidence Interval
Sensitivity	95.1% (137/144)	90.3-97.6
Specificity	97.4% (113/116)	92.7-99.1

Table 21: Summary of Theranos HSV-1 IgG Assay Performance in Pregnant Women Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	188	1	4	193
	Equivocal	0	1	0	1
	Negative	2	2	100	104
	Total	190	4	104	***298***

	Point Estimate	95% Confidence Interval
Sensitivity	97.9% (188/192)	94.8-99.2
Specificity	95.2% (100/105)	89.3-98.0

CDC Panel Testing

The objective of this study was to demonstrate agreement of the Theranos HSV-1 IgG Assay with the CDC panel. A panel of well characterized serum samples (n=100) was obtained from the U.S. Centers for Disease Control and Prevention (CDC). The CDC sample panel was tested with the HSV-1 IgG Assay and the results obtained by Theranos were sent to the CDC for confirmation. The panel consisted of 54 positives and 46 negatives. The Theranos HSV-1 IgG Assay demonstrated 100% agreement with the results provided by the CDC.

Low Prevalence Population

Serum samples were collected from a low prevalence population: Individuals who are not sexually active, and without a recent or current sexually transmitted disease (Hepatitis, Syphilis, HIV, HPV, Trichomonas, Chlamydia, Gonorrhoeae) as determined in an interview. Performance of the assay on this population is summarized in Table 22. The Samples were obtained from multiple specimen sources covering 10 US states and Mexico.

Table 22: Summary of Theranos HSV-1 IgG Assay Performance with Low Prevalence Population

		Reference Method			
		Positive	Equivocal	Negative	Total
Theranos HSV-1 IgG Assay	Positive	32	0	0	32
	Equivocal	0	0	0	0
	Negative	0	1	49	50
	Total	32	1	49	***82***

	Point Estimate	95% Confidence Interval
Sensitivity	97.0% (32/33)	84.7-99.5
Specificity	100% (49/49)	92.7-100

CONCLUSIONS

The results of the analytical and clinical performance studies submitted in this premarket notification are complete and demonstrate that the Theranos HSV-1 IgG Assay meets the established specifications necessary for consistent performance during intended clinical use. The results support a decision that the Theranos Herpes Simplex Virus-1 (HSV-1) IgG Assay is substantially equivalent to the predicate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K143236

Device Name
Theranos Herpes Simplex Virus-1 IgG Assay

Indications for Use *(Describe)*
The Theranos™ HSV-1 IgG Assay is a chemiluminescent immunoassay intended for the qualitative detection of IgG antibodies to herpes simplex virus type 1 (HSV-l) in human serum, in K2-EDTA anticoagulated human plasma from venous blood, and in human fingerstick K2-EDTA anticoagulated whole blood obtained with the Theranos Capillary Tubes and Nanotainer Tubes. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-l infection. The predictive value of positive and negative results depends on the population`s prevalence and the pretest likelihood of HSV-1.

The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates and immunocompromised patients.

The Theranos HSV-1 IgG Assay is for use with the Theranos System which performs automated sample processing steps and result analysis.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

THERANOS, INC.
BRAD ARINGTON
ASSOCIATE DIRECTOR, REGULATORY
1701 PAGE MILL ROAD
PALO ALTO, CA 94304

July 7, 2015

Re: K143236
Trade/Device Name: Theranos Herpes Simplex Virus-1 IgG Assay
Regulation Number: 21 CFR 866.3305
Regulation Name: Herpes simplex virus serological assays
Regulatory Class: II
Product Code: MXJ
Dated: June 29, 2015
Received: June 30, 2015

Dear Mr. Arington:

This letter corrects our substantially equivalent letter of July 2, 2015.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Sally A. Hojvat -S

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
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Enclosure